REMARKS

In response to the Office Action mailed August 15, 2008, Applicants have amended claims 1 and 25. Claims 5 and 16 have been canceled and no new claims have been added. It is urged that support for all the above amendments may be found throughout the specification as originally filed, for example on page 6, lines 1-2 and original claim 16. No new matter has been added. The above amendments are not to be construed as acquiescence with regard to the Examiner's rejections and are made without prejudice to prosecution of any subject matter removed or modified by this amendment in a related divisional, continuation or continuation-in-part application. Following the amendments, claims 1, 7, 10-13, 15, and 25 are under examination in the application. Favorable reconsideration of the subject application is respectfully requested in view of the above amendments and the following remarks.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph, Written Description, First Rejection

Claims 1, 5, 7, 13, 15, 16, stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement for allegedly claiming subject matter which was not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner contends that Applicant was not in possession of the bacterial species *Listeria monocytogenes* at the time of filing the instant application.

Applicant respectively traverses this basis of rejection and submits that the skilled artisan would readily understand that Applicant was in possession of the claimed invention at the time the application was filed. Nonetheless, Applicant, without acquiescing to any rejection and solely in a good faith effort to expedite prosecution, has amended claim 1 to remove the recitation of *Listeria monocytogenes*. Accordingly, Applicant submits that this basis for rejection has been rendered moot and may be properly withdrawn.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph, Written Description, Second Rejection

Claims 1, 5, 7, 13, 15, 16, and 25 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement for allegedly claiming subject matter which was not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner asserts that Applicant's disclosure allegedly fails to identify which aminopeptidases are specifically absent from the target microorganisms, but are present in all non-target microorganisms.

Applicant respectively traverses this basis of rejection and submits that the skilled artisan would readily understand that Applicant was in possession of the claimed invention at the time the application was filed. Applicant, without acquiescence to any rejection and solely in a good faith effort to expedite prosecution, has amended claims 1 and 25 to recite wherein the growth supporting medium further comprises antibiotics to suppress the growth of non-target microorganisms. Applicant has further amended claims 1 and 25 to recite wherein the target microorganism is *Campylobacter*. Support for these amendments can be found throughout the as-filed specification, for example, on page 6, lines 1-2, and in original claim 16. No new matter has been introduced by way of these amendments.

The Examiner contends that the claimed compositions comprise a substrate for an aminopeptidase, wherein the aminopeptidase is substantially absent from the target microorganism and is cleaved by substantially all non-target microorganisms and that the phrase "all non-target microorganisms" is interpreted to include any and all microorganisms that are not the target microorganism. The Examiner concludes that in order to satisfy this limitation, the composition must include a substrate for an aminopeptidase which is absent from the claimed target microorganisms but is present in all other microorganisms. Applicant respectfully disagrees.

The instant claims are drawn to a composition for detecting a target microorganism in a sample, wherein the microorganism is *Camplyobacter*. Further, the presently claimed compositions comprise a growth-supporting medium for the specific enrichment of a target microorganism, wherein said growth-supporting medium contains antibiotics to suppress the growth of non-target microorganisms in the sample. Applicant respectfully submits and the Examiner has acknowledged that *Campylobacter* and Gram positive bacteria lack L-alanine aminopeptidase activity, while all other Gram negative bacteria have this activity. Applicant respectfully points out that suppressing the growth of the non-target microorganisms in the sample using antibiotics would include suppressing the growth of Gram positive microorganisms, as such methods were known in the art at the time of filing the instant application (see *Microbiology: Principles and Applications*. Creager, Black, and Davison, Prentice Hall publishers, 1990; Corry et al., 1995; and U.S. Patent No. 5,891,709; copies included for your convenience). Thus, in the claimed composition, an L-alanine aminopeptidase substrate is absent in the target microorganism (e.g., *Campylobacter*) and present in substantially all non-target microorganisms in the sample (e.g., other Gram-negative bacteria).

Thus, Applicant respectfully submits that one having ordinary skill in the art would reasonably conclude that Applicant was in possession of the presently claimed aminopeptidase substrates. Accordingly, Applicant submits that the presently claimed invention satisfies the written description requirement of section 112 and respectfully requests reconsideration and withdrawal of this basis for rejection.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph, Enablement

Claims 1, 5, 7, 10-13, 15, 16 and 25 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. Specifically, the Examiner asserts that the as-filed specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with the claims.

Applicant respectfully traverses these bases for rejection and submits that the as-filed specification provides ample disclosure and guidance to practice the entire claimed scope of the invention. As noted above, claims 1 and 25 to recite wherein the growth supporting medium further comprises antibiotics to suppress the growth of non-target microorganisms and wherein the target microorganism is *Campylobacter*.

The Examiner has acknowledged that the presently claimed compositions are enabled for detecting a pure culture of *Campylobacter*, but are not enabled for the production and use of compositions for detecting any target microorganism in any sample, or even for detecting *Campylobacter* in a mixed sample, or for differentiating between *Campylobacter* and any Gram-positive bacteria. Applicant respectfully disagrees.

Applicant has demonstrated aminopeptidases that are absent in the target bacteria were known in the art; thus, the skilled artisan would only need to determine the aminopeptidase activity present in substantially all the bacteria in the sample, (*i.e.*, not all known bacteria in existence). Furthermore, Applicant submits that in most samples where contamination of biological samples is a concern (*e.g.*, poultry, beef), the skilled artisan is keenly aware of the type of bacterial pathogens that predominantly contaminate the sample (*e.g.*, poultry – *Campylobacter*; beef – *E. coli*). Moreover, Applicant respectfully notes that one having ordinary skill in the art would recognize that no singular sample exists, wherein all non-target bacteria could be present, because different species of bacteria require different temperatures, pHs, osmolarities, and require different nutrients in order to grow.

The Examiner contends that when the presently claimed assay is used to identify *Campylobacter*, Gram-positive bacteria will give rise to the same signals as presumptive and confirmation indicators as *Campylobacter*, and therefore, the presently claimed compositions are only enabled for pure *Campylobacter* cultures. However, as noted in the foregoing response to the written description rejection, the claimed compositions comprise a growth-supporting medium for the specific enrichment of a target microorganism, wherein said growth-supporting medium contains antibiotics to suppress the growth of non-target microorganisms in the sample.

Thus the skilled artisan would use a growth supporting media for the specific enrichment of *Campylobacter*, which would exclude the growth of Gram-positive organisms, in combination with antibiotics to suppress the growth of non-target microorganism in the sample, including, but not limited to Gram positive bacteria, as such media are commonly known to one of ordinary skill in the microbiological arts (see, see *Microbiology: Principles and Applications*. Creager, Black, and Davison, Prentice Hall publishers, 1990; Corry et al., 1995; and U.S. Patent No. 5,891,709).

In addition, Applicant has provided two examples of a reduction to practice of the presently claimed compositions. Examples 1 and 2 and Figure 1 provide evidence that the number of false positives in the poultry wash samples are negligible (*i.e.*, most colonies are target microorganisms, and not Gram-positive bacteria or other non-target bacteria) by demonstrating that the number of *Campylobacter* in said samples is the same, whether determined by the Applicant's method or the method approved by the U.S. government, wherein the presence of *Campylobacter* is confirmed by agglutination assays. Thus, the growth of non-target microorganisms is effectively suppressed in exemplified embodiments of the presently claimed compositions. Applicant has also produced a commercial embodiment for the specific detection of *Campylobacter* in mixed bacterial samples (http://www.biocontrolsys.com/products/simcamp.html; a copy of the document is enclosed for your convenience). Currently, this product is used by customers for the detection of *Campylobacter*. Accordingly, the Examiner's assertion that the present claims are not enables is simply not true.

Accordingly, Applicant submits that in view of the foregoing remarks and amendments, the skilled artisan would readily be able to practice the full scope of the presently claimed invention without undue experimentation. Reconsideration and withdrawal of this basis for rejection is respectfully requested.

Claim Rejection under 35 U.S.C. § 103(a)

Claims 1, 5, 7, 10-13, 15, 16, and 25 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Manafi et al. (J. Applied Bacteriology, 1990) in

view of Molina et al. (Enfermedades Infecciosas y Microbiologia Clinica, 1991) and Tuompo et al. (U.S. Patent No. 5,420,017). Specifically, the Examiner contends that Manafi et al. disclose a method and composition for detecting Gram-negative bacteria in a sample using AAMC. Further, the Examiner contends that Tuompo et al. disclose a method and kit for detecting microorganisms in a sample using tetrazolium red. The Examiner alleges that the skilled artisan would find it obvious to combine the teachings of Manafi et al. with Tuompo et al. to arrive at the presently claimed composition.

Applicant traverses this basis of rejection and respectfully submits that the Action fails to establish a *prima facie* case of obviousness against the presently claimed invention because the references do not teach or suggest each and every element of the claims. "To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Furthermore, Manafi et al. and Molina et al. actually teach away from using an L-alanine aminopeptidase substrate to detect Campylobacter, and in contrast use L-alanine aminopeptidase substrates to detect other Gram negative bacteria. Accordingly, the Action fails to provide a sufficient basis for one having ordinary skill in the art to predictably arrive at the presently claimed invention with any reasonable expectation of success, and thus, the Action fails to establish a *prima facie* case of obviousness against the presently claimed invention.

The Examiner alleges that the skilled artisan would find it *prima facie* obvious to modify the composition of Manafi et al., which includes AAMC in an agar plate, to further include tetrazolium red, as taught by Tuompo et al. However, Applicant respectfully submits the Action has failed to account for each and every element of the presently claimed composition and that Manafi et al., Molina et al., and Tuompo et al., collectively fail to teach or suggest the use of a growth-supporting medium comprising antibiotics to suppress non-target microorganisms in the sample. At a minimum, it must be demonstrated that the cited references provide a sufficient basis to predictably arrive at the presently claimed invention, and even assuming, *arguendo,* that the cited references teach each claim feature, the Examiner must provide an explicit, apparent reason to combine these features in the fashion claimed by the Applicant with a reasonable expectation of success. *See KSR v. Teleflex, Inc.*, No. 04-1350 at 4, 14 (U.S. Apr. 30,

2007) ("A patent composed of several elements is not proved obvious merely by demonstrating that each element was, independently, known in the prior art"). In the instant case, the Examiner has not provided any rationale to support the inclusion of antibiotics in the presently claimed compositions. Thus, this Action fails to support a *prima facie* case of obviousness against the presently claimed invention.

The Examiner further alleges that including a viability marker in the agar plate of Manafi et al. would provide an additional confirmation means of bacterial viability. Applicant respectfully submits that one of ordinary skill in the art would not include a viability dye for bacteria cultured on the **non-selective** media of Manafi et al.. Inclusion of the dye in the embodiment contrived by the Examiner would be redundant and would fail to provide any further information regarding the number of viable bacteria, as such information is already gleaned from colony formation. For example, the embodiment contrived by the Examiner (non-selective media) would function exactly the same in the presence and absence of tetrazolium red. In contrast, eliminating tetrazolium red would prevent the presently claimed composition from detecting false positives. Thus, Applicant submits that tetrazolium red functions differently in the Examiner's contrived composition in comparison to the presently claimed compositions.

A rationale to support a conclusion that a claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions (emphasis added), and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 USPQ2d 1385, 1395 (2007); *Sakraida v. AG Pro, Inc.*, 425 U.S. 273, 282, 189 USPQ 449, 453 (1976); *Anderson's-Black Rock, Inc. v. Pavement Salvage Co.*, 396 U.S. 57, 62-63, 163 USPQ 673, 675 (1969); *Great Atlantic & P. Tea Co. v. Supermarket Equipment Corp.*, 340 U.S. 147, 152, 87 USPQ 303, 306 (1950).

Furthermore, in the presently claimed composition, an aminopeptidase substrate which is absent in the target microorganism, is used to detect the presence of the target microorganism. Neither Manafi et al., Molina et al., nor Tuompo et al., provide a sufficient basis for the skilled artisan to predictably practice the detection of

Campylobacter based upon the absence of L-alanine aminopeptidase activity with any reasonable expectation of success. In contrast, the entirety of the Manafi et al. reference and the abstract of the Molina et al. reference teach away from the presently claimed compositions for detecting *Campylobacter,* and in contrast teach using the L-alanine aminopeptidase substrates to detect other Gram negative bacteria. Applicant submits that using an aminopeptidase substrate to detect a microorganism that lacks the aminopeptidase activity for that substrate counters conventional wisdom of one having ordinary skill in the art, and is thus, nonobvious. The skilled artisan would not seek to detect a microorganism based on its lack of a particular enzymatic activity, as demonstrated in the Examiner's cited references. Applicant respectfully submits that "[t]he totality of the prior art must be considered, and proceeding contrary to accepted wisdom in the art is evidence of nonobviousness." *In re Hedges*, 783 F.2d 1038, 228 USPQ 685 (Fed. Cir. 1986).

Accordingly, Applicant submits that the Examiner has failed to establish a *prima facie* case of obviousness against the presently claimed invention. The Action fails to provide a sufficient basis for one having ordinary skill in the art to predictably arrive at the presently claimed invention with any reasonable expectation of success, and in fact, teaches away from the presently claimed composition to detect *Campylobacter.* Reconsideration and withdrawal of this basis of rejection is respectfully requested.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are now believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

/William T. Chistiansen/
William T. Christiansen, Ph.D.
Registration No. 44,614

WTC:jto

Enclosures:

Microbiology: Principles and Applications. Creager, Black, and Davison, Prentice Hall publishers, 1990
Corry et al., 1995
U.S. Patent No. 5,891,709

701 Fifth Avenue, Suite 5400
Seattle, Washington 98104
Phone: (206) 622-4900
Fax: (206) 682-6031

1272475_1.DOC

MICROBIOLOGY
Principles and Applications

Joan G. Creager
Marymount University

Jacquelyn G. Black
Marymount University
Northern Virginia Community College

Vee E. Davison
Epidemiology Laboratory,
USAF School of Aerospace Medicine

Prentice Hall
Englewood Cliffs, New Jersey 07632

Library of Congress Cataloging-in-Publication Data

Creager, Joan G.
Microbiology: principles and applications/Joan G. Creager, Jacquelyn G. Black, Vee E. Davison.
p. cm.
Includes bibliographical references.
ISBN 0-13-584251-4
1. Medical microbiology. I. Black, Jacquelyn G. II. Davison, Vee E. III. Title.
QR46.C877 1990
616'.01—dc20 89-22992
CIP

Editorial/production supervision: Eleanor Henshaw Hiatt; Rachel J. Witty, Letter Perfect, Inc.
Development editor: Lee Marcott, Dan Schiller
Interior design: Jayne Conte; Anne T. Bonanno
Manufacturing buyer: Paula Massenaro
Cover design: Bruce Kenselaar
Page makeup: Meryl Poweski
Photo research: Margaret B. Ober; John Schultz
Photo editor: Lorinda Morris-Nantz
Cover photo: Alexander Tsiaras/Science Source/Photo Researchers, Inc.
Illustrators: William C. Ober, M.D.; Claire W. Garrison, R.N.; Vantage Art Inc.; Joel Ito/Melissa Turk & The Artist Network; Dennis Tasa

Photo credits begin on page 833 as a continuation of the copyright page.

Printed in the United States of America
10 9 8 7 6 5 4

0-13-584251-4

Prentice-Hall International (UK) Limited, *London*
Prentice-Hall of Australia Pty. Limited, *Sydney*
Prentice-Hall Canada Inc., *Toronto*
Prentice-Hall Hispanoamericana, S.A., *Mexico*
Prentice-Hall of India Private Limited, *New Delhi*
Prentice-Hall of Japan, Inc., *Tokyo*
Simon & Schuster Asia Pte. Ltd., *Singapore*
Editora Prentice-Hall do Brasil, Ltda., *Rio de Janeiro*

TABLE 6.5
Selected examples of diagnostic media

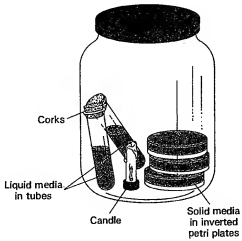
Medium	Organism(s) identified	Selectivity and/or Differentiation achieved
Brilliant green agar	*Salmonella*	Brilliant green dye inhibits gram-positive bacteria and thus selects gram-negative ones. Differentiates *Shigella* colonies (which do not ferment lactose or sucrose and are red to white) from other organisms that do ferment one of the sugars and are yellow to green.
Desoxycholate agar	Gram-negative enterics	Sodium desoxycholate inhibits gram-positive bacteria. Differentiates organisms that ferment lactose (red colonies) from those that do not (colorless colonies).
Eosin methylene blue agar	Gram-negative enterics	Medium partially inhibits gram-positive bacteria. Eosin and methylene blue differentiate among organisms: *E. coli* colonies have a metallic green sheen, *Enterobacter aerogenes* colonies are pink, indicating that they ferment lactose, and colonies of other organisms are transparent, indicating they do not ferment lactose.
Sodium tetrathionate broth	Enteric pathogens	Sodium tetrathionate inhibits normal inhabitants of the gut and enriches growth of certain pathogens, such as *Salmonella* and *Shigella*.
Triple sugar-iron agar	Gram-negative enterics	Used in agar slants (tubes cooled in slanted position) where differentiation is based on both aerobic surface growth and anaerobic growth in agar in base of tube. Medium contains glucose, sucrose, and lactose and a pH indicator, so relative use of each sugar can be detected.

which might not otherwise be present in sufficient numbers to allow it to be isolated and identified. For example, because *Salmonella typhi* organisms may not be sufficiently numerous in a fecal sample to allow positive identification, they are cultured on a medium containing the trace element selenium, which fosters growth of the organism. After incubation in the enrichment medium, the greater numbers of the organisms increase the likelihood of a positive identification.

Controlling Oxygen Content of Media

Obligate aerobes, microaerophiles, and obligate anaerobes require special attention to maintain oxygen concentrations suitable for growth. Most obligate aerobes obtain sufficient oxygen from nutrient broth or solidified agar medium, but some need more. Oxygen gas is bubbled through the medium with filters between the gas source and the medium to prevent contamination of the culture. Microaerophiles can be incubated in nutrient broth tubes or agar plates in a jar in which a candle is lit before the jar is sealed (Figure 6.18). (Scented candles should not be used because oils from them inhibit bacterial growth.) The burning candle uses oxygen from the air in the jar and adds carbon dioxide to it. When the carbon diox-

FIGURE 6.18 Microaerophiles are growing in culture tubes and on Petri plates in a sealed jar in which a candle burned until it was extinguished by carbon dioxide accumulation in the atmosphere of the jar. A small amount of oxygen remains.



ELSEVIER

International Journal of
Food Microbiology 26 (1995) 43–76

International Journal
of Food Microbiology

Culture media for the isolation of campylobacters

Janet E.L. Corry [a,*], D.E. Post [b], P. Colin [c], M.J. Laisney [c]

[a] *Department of Clinical Veterinary Science, University of Bristol, Langford, Avon, BS18 7DU, UK*
[b] *Unipath Limited, Wade Road, Basingstoke, Hants, RG24 8PW, UK*
[c] *National Centre for Veterinary and Food Studies (CNEVA), Central Laboratory for Poultry and Pig Research (LCRAP), Beausemaine, B.P. 53-22440, Ploufragan, France*

Abstract

The history of the development of selective media for isolation of campylobacters, including the rationale for choice of selective agents is described. Developments have included modifications to allow incubation at 37°C instead of 42 or 43°C and changes in the types and concentrations of antibiotics in order not to inhibit organisms such as *Campylobacter upsaliensis*, *C. jejuni* subsp. *doylei* and some strains of *C. coli* and *C. lari*. When examining foods, plating media originally developed for isolation from faeces are normally used, sometimes after liquid enrichment. Most of the media include ingredients intended to protect campylobacters from the toxic effect of oxygen derivatives. Most commonly used are lysed or defibrinated blood; charcoal; a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP); and haemin or haematin. To date no medium includes an indicator system – for instance a pH indicator to show whether colonies produce acid or alkali from particular substrates. The manner in which liquid enrichment media are used has been modified for food samples to avoid inhibitory effects on sublethally damaged cells by toxic components in the formula. This is done by a preliminary period of incubation at reduced temperature and sometimes by delayed addition of antibiotics. Expensive and time-consuming methods have been proposed to achieve a microaerobic atmosphere while using liquid enrichment media. To date there is no generally accepted 'standard' method of isolating campylobacters from food.

Keywords: *Campylobacter*; *Arcobacter*; Enrichment media; Plating media; Membrane filtration; Foods; Water

* Corresponding author.

SSDI 0168-1605(95)00044-7

Campylobacters were originally classified within the genus *Vibrio*, but differ from vibrios in a number of respects, particularly in their DNA base composition and their ability to grow under conditions of reduced oxygen tension. They are Gram-negative, oxidase-positive, curved or spiral rod-shaped bacteria 0.2–0.5 μm wide and 0.5–8 μm long. They possess one polar flagellum, which gives them a very characteristic 'cork-screw' motility. Survival of campylobacters is poor under most conditions and particularly in dry, relatively warm and aerobic situations. As cultures lose their viability the rod-shape changes to a coccoid form which has been described as 'viable but non-culturable'. This is because, although these forms cannot be grown using normal media, at least some have been shown to be capable of initiating infections in animals (e.g. Jones et al., 1991)

The genus *Campylobacter* has recently been subdivided into three related genera: *Campylobacter*, which contains the species *C. fetus*, *C. jejuni*, *C. coli*, *C. lari* (NARTC, formally known as 'nalidixic acid resistant thermophilic campylobacters'), *C. hyointestinalis*, *C. upsaliensis*, *C. mucosalis*, *C. concisus*, *C. sputorum*; *Helicobacter* which contains *H. pylori* (previously *C. pyloridis*, responsible for human gastric infection and gastric ulcers) *H. cinaedi*, *H. fennelliae* and *H. mustelae*; *Arcobacter* which contains *A. cryaerophilus*, *A. butzleri*, *A. nitrofigilis* and *A. skirrowii* (Vandamme et al., 1991, 1992; Vandamme and Goossens, 1992; Tenover and Fennell, 1992). Table 1 lists the most important species of this group, which are associated with human and/or animal diarrhoea together with their important characteristics.

Selective media were originally designed to isolate *C. jejuni* from faeces, by use of a cocktail of antibiotics in a rich basal medium and exploiting the ability of this organism to grow at 42 or 43°C (Butzler and Skirrow, 1979). Later *C. coli* and *C. lari* (formerly *C. laridis*) were distinguished, although many workers either failed to differentiate between these three species when monitoring the functioning of selective media or only tested for *C. jejuni* (e.g. Park et al., 1981; Rosef, 1981; Acuff et al., 1982a,b; Bolton et al., 1982; Goossens et al., 1983; Martin et al., 1983; Wesley et al., 1983; Barot and Bokkenhauser, 1984; Stern et al., 1984; Beuchat et al., 1985; Furlanetto et al., 1991; Holler, 1991; Jacob and Stelzer, 1992; Stern et al., 1992; Stern and Line, 1992; Scotter et al., 1993).

There is evidence that some strains of *C. coli* and even a few strains of *C. jejuni* are likely to have been missed due to their sensitivity to cephalothin (Brooks et al., 1986; Ng et al., 1985, 1988). More recent work has indicated that other species of campylobacter of importance in human intestinal disease will not be isolated by the usual selective media because of their susceptibility to the antibiotics used in most media e.g. *C. upsaliensis* (Warmsley and Karmali, 1989; Goossens et al., 1990a). In addition a closely related group of organisms unable to grow at 42 or 43°C, the normal isolation temperature, and recently reclassified as *Arcobacter* rather than *Campylobacter* spp. on the basis of their aerotolerance and various genotypic tests (Vandamme et al., 1991, 1992), are increasingly being implicated as important causes of human gastro-intestinal disease (Patton et al., 1989; Lastovica et al., 1989; Kiehlbauch et al., 1991; Taylor et al., 1991). At present, therefore, *Arcobacter* species and *C. upsaliensis* are unlikely to be detected as causes of

human (or animal) diarrhoea, neither would they be detected in routine examination of human or animal faeces, foods, water or environmental specimens unless membrane filtration and non-selective media at 37°C are used. Recently, however, a modification to blood-free selective agar has been described which enables *C. upsaliensis* to be isolated, thus removing the practical difficulties of the filtration method (Aspinall et al., 1993). Between 1989 and 1991 only 15% of thermophilic campylobacters reported as causes of human diarrhoea in England and Wales were identified to species level. Of these 89–93% were *C. jejuni* and 7–10% were *C. coli* or *C. lari*, with 0.2–0.4% other species of campylobacter (Pearson and Healing, 1992). However, Goossens et al. (1990b) reported *C. upsaliensis* to be the cause of 13% of campylobacter diarrhoea cases in Belgium. *C. jejuni* subsp. *doylei* is another strain implicated as a cause of diarrhoea that frequently does not grow at 42°C and is sensitive to cephalothin (Taylor et al., 1991; Bolton et al., 1992). These considerations should be borne in mind when reading this review, which will be concerned almost exclusively with the thermophilic campylobacters.

The media used for isolating campylobacters from foods and water have been derived from those first developed for the isolation of campylobacters from faeces. In some cases the same plating media are still used for both purposes. In line with techniques developed for isolating other pathogens, such as salmonellas, from foods and other environments, liquid (enrichment) media have been developed, and also pre-enrichment media, intended to aid recovery of sublethally damaged campylobacters. Incubation has usually been carried out at 42 or 43°C.

Besides a variety of selective agents, almost all of which are antibiotics, media for campylobacter usually contain ingredients to neutralise the toxic effects of substances that form in the presence of oxygen and light. In addition, almost all workers have found it necessary to incubate plates in an atmosphere of about 5–7% oxygen, 10% carbon dioxide and 80% nitrogen and/or hydrogen. This can be achieved by using a bottled gas mixture of those proportions, by using the appropriate gas-generating envelope or by replacing two thirds of the atmosphere with a mixture of either nitrogen or hydrogen plus 5–15% carbon dioxide (Skirrow et al., 1982). Skirrow et al. (1991) reported that the presence of hydrogen at not less than 7% improved the primary isolation of *C. jejuni* from faeces. Candle jars have been reported to be successful for incubation of plates of Butzler's medium Virion, provided 37°C is used (Goossens et al., 1983). Ribeiro et al. (1985) compared growth of *C. jejuni*, *C. coli* and *C. lari* in atmospheres generated by burning ethanol (methylated spirit) rather than a candle (paraffin wax) and found higher colony counts and larger colonies using ethanol. Colony counts for *C. lari* were particularly low when using candles. Similar comparisons by Skirrow et al. (1987) confirmed these results. Best results were obtained when the spirit was burned in a 90 mm diameter Petri dish with the jar not more than half full with dishes. Pennie et al. (1984) found that a satisfactory atmosphere could be obtained by using a mixture of grade 0 steel wool, previously soaked in cupric sulphate solution and an Alka-Seltzer (sodium bicarbonate) tablet in water, all placed in a plastic bag! Jacob and Stelzer (1992) reported that this method worked well.

Recent work by Jones et al. (1993) has shown that *C. jejuni* may adapt to grow

Table 1a
Diagnostic features of *Campylobacter* and *Arcobacter* species causing illness in man

	Growth aerobically at 30°C or 36°C	Growth microaerobically			Catalase	Oxidase	Nitrate reduction	Cephalothin 30 μg	Nalidixic acid 30 μg
		25°C	37°C	42°C					
C. jejuni									
subsp. *jejuni*	−	−	+	+	+	+	+	R	S
subsp. *doylei*	−	−	+	V	D	+	−	S	S
C. coli	−	−	+	+	+	+	+	R	S
C. fetus	−	+	+	W	+	+	+	S	R
C. lari	−	−	+	+	+	+	+	R	R
C. upsaliensis	−	−	+	D	W	+	+	S	S
C. hyointestinalis	−	D	+	D	+	+	+	S	R
Arcobacter butzleri	+	+	+	−	W	+	+	R	S
A. cryaerophilus	+	+	V	−	W	+	V	R/S	S
A. nitrofigilis	+	+	W	−	+	+	+	R/S	S
A. skirrowii	+	+	+	V	+	+	+	R/S	S

V, 50% positive; W, negative or weakly positive; D, some +, some −; R, resistant; S, sensitive; TSI, triple sugar iron medium.
From: Barrett et al., 1988; Lastovica et al., 1989; Patton et al., 1989; Warmsley and Karmali, 1989; Boudreau et al., 1991; Kiehlbauch et al., 1991; Tenover and Fennell, 1992; Vandamme et al., 1991, 1992; Vandamme and Goossens, 1992.

Table 1b

	Hydrogen sulphide production in TSI	Indoxyl acetate hydrolysis	Hippurate hydrolysis	Urea hydrolysis	Growth in 1% glycine	Growth on MacConkey agar	Growth on Campy-BAP agar (BBL)	Growth on Campy-CVA (Campy-BAP (BBL) minus polymyxin)
C. jejuni								
subsp. *jejuni*	−	+	+	−	+	+	+	+
subsp. *doylei*	−	+	+	−	+	?	?	?
C. coli	W	+	−	−	+	+	+	+
C. fetus	−	−	−	−	W	+	?	?
C. lari	−	−	−	−	+	+	+	+
C. upsaliensis	−	+	−	−	+	+	?	?
C. hyointestinalis	+	−	−	−	+	+	?	?
Arcobacter butzleri	−	+	−	−	+	D	−	+
A. cryaerophilus	−	+	−	−	+	−	−	+
A. nitrofigilis	−	W	-	V	−	W	−	−
A. skirrowii	−	+	−	−	+	−	?	?

Table 2
Most widely used isolation media for thermophilic campylobacters

Plating Media
Skirrow (Skirrow, 1977; see also Baird et al., 1987)
Butzler (Lauwers et al., 1978)
Campy BAP (Blaser et al., 1978)
Preston agar (Bolton and Robertson, 1982; see also Baird et al., 1987)
Butzler (Virion) (Goossens et al., 1983)
Butzler (Oxoid) (Goossens et al., 1983)
mCCD agar (Bolton et al., 1984 modified according to Hutchinson and Bolton, 1984; see also Baird et al., 1987)
Karmali agar (Karmali et al., 1986)
Semi-solid medium (Goossens et al., 1989)
Enrichment Media
Preston broth (Bolton et al., 1982; see also Baird et al., 1987)
Doyle and Roman broth (1982)
VTP FBP broth (a modification by Lovett et al., 1983, of the medium of Park et al., 1981)
mCCD broth (Bolton et al., 1984, modified according to Hutchinson and Bolton, 1984)
Park and Sanders broth (1991)
Exeter broth (De Boer and Humphrey, 1991)
Hunt and Radle broth (Hunt, 1992)

in a normal air atmosphere. The implications of this observation are not yet clear, but may explain to some extent the 'viable but non-culturable' phenomenon. Fraser et al. (1992) reported that, provided the relative humidity was 99%, normal air containing 10% carbon dioxide gave larger colonies of *C. jejuni* and *C. coli* than the conventional gas mixture. However at 95% RH growth was poor. Other workers have reported that *C. jejuni*, as evidenced by its colonial morphology, is sensitive to the moisture level in plating media (Buck and Kelly, 1981), although changes in colonial morphology could also be due to inhibitory oxygen derivatives formed during storage and drying of plates.

No campylobacter media with indicator systems for identification of presumptive campylobacter colonies have yet been developed. Experienced workers generally claim to be able to recognise the organism by the typical appearance of colonies – flat, glossy and effuse, thinly spreading if the agar is moist. However, colonies are sometimes atypical, especially if plates are rather dry, so Gram staining of oxidase-positive colonies and examination by microscope for the characteristic morphology is advisable.

The number of formulations proposed for the isolation of thermophilic campylobacters probably exceeds that for any other group of bacteria, especially if one considers that all have been published since 1972 and almost all since 1977. Table 3 summarises many of these. Before the medium of Dekeyser et al. (1972) was available, isolation of 'related Vibrio' (as campylobacters were then known) depended on the use of membrane filtration followed by subculture onto nutritionally

rich blood agar. The method of Dekeyser and co-workers used a combination of centrifugation, filtration through a 0.65 μm membrane filter and plating of the filtrate onto a selective agar.

Even if all the campylobacter media formulae had been published with a full description of the rationale used in their development, there would be insufficient space to consider them in detail. This review will examine the basic principles the media have in common and then concentrate on the details of the most widely used ones. The commonly used plating and liquid media are listed in Table 2. Filtration methods have frequently been used to concentrate campylobacters in samples of water (Bolton et al., 1982; 1987; Ribeiro and Price, 1984; Rosef et al., 1987; Stelzer et al., 1988; Brennhovd et al., 1992). Filtration is sometimes followed by liquid enrichment, sometimes by direct plating onto selective agar. Centrifugation has also been used when examining food samples (Lovett et al., 1983).

1. Basal media

Few publications provide an explanation for the choice of basal medium and many have been used. Although campylobacters will grow in relatively simple media such as nutrient agar, most workers have used basal media developed for other fastidious capnophilic or anaerobic pathogens such as brucella or thioglycollate medium, Columbia or blood agar base and Müller Hinton broth. However, Bolton and Robertson (1982) chose nutrient broth no. 2, an unsophisticated medium, as the basis for their Preston media because it contained less thymidine, an antagonist to the activity of the selective agent trimethroprim, used in most selective solid and liquid media (see Table 3).

2. Blood

Most solid media incorporate blood at levels between 5 and 15%. Some media use defibrinated blood from various animals and others lysed horse blood. Only one uses lysed sheep blood (Stelzer and Jacob, 1992). Skirrow et al. (1982) stated that lysed horse blood was needed to neutralise trimethoprim antagonists which are present in most media. Many media which incorporate trimethoprim do not contain lysed horse blood although blood of some type is usually present. The media of Martin et al. (1983) and Goossens et al. (1989), neither of which contain blood, have the highest concentrations of trimethoprim. Presumably this is necessary to counteract the thymidine. Blood is also thought to be active in neutralising toxic oxygen derivatives (Juven et al., 1985; Weinrich et al., 1990).

3. FBP and other bloodless supplements

A combination of ferrous sulphate ($FeSO_4 \cdot 7H_2O$), sodium metabisulphite and sodium pyruvate (FBP), each at 0.25 or 0.5 g per litre was suggested by George et

Table 3
Formulation of liquid and solid selective media for thermophilic campylobacters (concentrations in mg per litre unless otherwise stated)

Medium name/ reference, Other details	Basal medium	Antioxygen system	Cephalosporins	Trimethoprim	Polymyxin B or Colistin (C) in iu	Vancomycin or Teicoplanin (T)	Rifampicin	Novobiocin	5-Fluorouracil or Na-deoxycholate (D)	Bacitracin in iu	Antifungals
Dekeyser A [1]	TA	15% S			10000			5		25000	50 CY
Skirrow A [2,a]	BAB	7% LH		5	2500	10					
Blaser A [3]	BA	10% S		5	2500	10		2			
Butzler A [4]	TB	10% S	15 cthin		10000 (C)			5		25000	50 CY
CAMPY-BAP A [5]	BA	10% S	15 cthin	5	2500	10					2 AM
Blaser-Wang A [6]	BAB	7% LH	15 cthin	5	2500	10					2 AM
C-2 A [7]	BA	5% H/FBP	5 cthin	5	8000	10					
C-3 A [7]	BA	5% H/FBP	1 cthin	5	2500	10					
Park (1981) B [8]	BB	–		4	8000	8					
BU40 B [9]	TB	10% S	15 cthin		40000 (C)			5		25000	50 CY
Rosef B [10,b]	NB	–		5	2500	10					
Preston A [11(a)]	NB	5% LH		10	5000		10				100 CY
Preston B [11(b)]	NB	5% LH/FBP		10	5000		10				100 CY
Doyle-Roman B [12, c]	BB	7% LH		5	20000	15					50 CY
Christopher B+A [13]	BB	0.5g P	15 cthin	5	2500	10					2 AM
FBP-AM B/A [14]	BB/A	FBP (B) 5% DH (A)	15 cthin	5	2500	10					2 AM
Lander B [15]	VI	5g C		10	50000	40			100		100 CY
Park-Stankiewicz B [16,d]	BB	2g F, 0.25g B, 0.5g P	30 cthin	4	3 mg (C)	8					
Butzler medium Virion A [17]	CA	5–7% S	15 czone		10000 (C)		10				2 AM
Butzler medium Oxoid A [18]	CA	5–7% S	15 czolin		10000 (C)	5				25000	50 CY
Modified Park (1981) B [19]	BB	FBP		7.5	5000	15					
CEB B [20]	BB	–	32 czone	32					333		
VTP-FBP B [21]	BB	FBP, 5% LH		10	5000	20					
CAK A [22]	GA	7% H	15 cthin								1 AM
Rosef-Kapperud A [23]	GA, IVx	–	15 cthin		10000 (C)						25000 iu NY
ATB [24,e]	T; YE, NaCl	FBP, HT	6.25 codin		20000		25				
CCD A [25,f]	NB	4g C, 0.25g F, 0.25g P	10 czolin						1000 (D)		
CCD B [25,f]	NB	4g C, 0.25g FBP	10 czolin						1000 (D)		

mCCD A [26,i]	NB	4g C, 0.25g F, 0.25g P	32 czone					1000 (D)	10 AM
Doyle-Roman B [27,g]	BB								
Steele-McDermott [28]	–	10%S + FBP		5	2.9 mg (C)	10			
Waterman A [29]	BAB	5% DH		5	2500		10		100 CY
Waterman B [29]	TB	5% DH		20	10000				
Virion [30]	CA	5–7% S	30 czone				10		2 AM
Exeter A + B [31,h]	NB	5% LH, 0.5g F, 0.2g B, 0.2g P	15 czone	10	4 mg (C)	10 [k]	10 [k]		
Karmali A [32,i]	CA	4g C, 0.32g HT, 0.1g P	32 czone						
CAR B/A [33]	BA/BB	FBP, 3% LH	32 czone				10		2.5 AM
Exeter A + B [34]	NB	FBP, 5% LH	15 czone		4 mg		10		2 AM
SSM B + A [35]	MHB + 4 g/l A	–	30 czone	50					
Bolton [36,j]	A	5% LH, 0.1g HE, 0.5g P, 0.5g B	20 czone			20			50 CY
Park-Sanders B [37]	BB	0.25 P, 1 g Na citrate, 5% LH	32 czone [l]	10		10			100 CY [l]
Hunt-Radle B [38]	NB, YE	FBP, 5% LH	15 czone [l,m]	12.5		10			100 CY or 2 AM
Campy Cefex A [39]	BA	0.5 g F, 0.2 g B, 0.5 g P, 5% LH	33 czone						200 CY
CAT A [40,f]	NB [f]	4g C	8 czone			4 (T)		1000 (D)	10 AM

Key: COLUMN 1. *References*: [1] Dekeyser et al., 1972; [2] Skirrow, 1977; [3] Blaser et al., 1978; [4] Lauwers et al., 1978; [5] Blaser et al., 1979; [6] Blaser et al., 1980; [7] Gilchrist et al., 1981; [8] Park et al., 1981; [9] Patton et al., 1981; [10] Rosef, 1981; [10a] Bolton and Robertson, 1982; [10b] Bolton et al., 1982; [12] Doyle and Roman, 1982; [13] Christopher et al., 1982; [14] Ehlers et al., 1982; [15] Lander, 1982; [16] Park and Stankiewicz, 1982; [17] Butzler et al., 1983; [18] Goossens et al., 1983; [19] Lovett et al., 1983; [20] Martin et al., 1983; [21] Park et al., 1983; [22] Rosef et al., 1983a; [23] Roseff and Kapperud, 1983; [24] Wesley et al., 1983; [25] Bolton et al., 1984b; [26] Hutchinson and Bolton, 1984; [27] Ray and Johnson, 1984b; [28] Steele and McDermott, 1984; [29] Waterman et al., 1984; [30] Goossens et al., 1986; [31] Humphrey, 1986b; [32] Karmali et al., 1986; [33] Welter et al., 1987; [34] De Boer and Humphrey, 1989; [35] Goossens et al., 1989; [36] Bolton, personal communication; [37] Park and Sanders, 1981; [38] Hunt and Radle, 1992 (cited by Stern and Line, 1992); [39] Stern et al., 1992; [40] Aspinall et al., 1993.

A, agar; B, broth; SSM, semi-solid selective motility medium; ATB, alkaline tryptone broth.

Other details (pH, growth factors, other additives): [a] pH 7.3–7.4; [b] yeast extract, resazurin; [c] Na succinate, cysteine HCl; [d] 3% calf serum; [e] 10 g bicine, pH 8.0; [f] casein hydrolysate; [g] modified protocol: add polymyxin after 6 h at 37°C for freeze-stressed campylobacters; [h] antibiotics added after 4 h at 37°C; [i] pH 7.4, 1 g Na deoxycholate; [j] α-ketoglutamic acid, $NaCO_3$.

COLUMN 2. Basal medium: TA, thioglycollate agar; TB, thioglycollate broth base; BA, brucella agar base; BB, brucella broth base; NB, nutrient broth; BAB, blood agar base; VI, veal infusion; CA, Columbia agar base; GA, gonococcus agar base; T, tryptose; MHB, Mueller-Hinton broth; YE, yeast extract; IVx, IsoVitalex (vitamin and nutrient mixture (BBL)); A, 10 g meat peptone, 5 g lactoalbumin hydrolysates, 5 g yeast extract, 5 g NaCl, 0.6 g $NaCO_3$.

COLUMN 3. Antiwygen system: S, sheep blood; H, horse blood; LH, lysed horse blood; DH, defibrinated horse blood; C, charcoal; F, ferrous sulphate ($FeSO_4 \cdot 7H_2O$); B, sodium metabisulphite ($NaHSO_3$); P, sodium pyruvate. At 0.5 g per litre (unless otherwise stated); HT, haematin; HE, haemin.

COLUMN 4. Cephalosporins: ethin, cephalothin; ezolin, cephazolin; czone; codin, cefsulodin. [k] 15 mg cefalexin instead of cephalothin in recipe of Butzler and Skirrow, 1979 fused without cephalosporins by Butzler et al., 1979. [l] added later. [m] 4–15 later.

[n] broth only. [o] agar only.

COLUMN 12. Antifungals: CY, cycloheximide (actidione); AM, amphotericin B; NY, nystatin.

al. (1978) and Hoffman et al. (1979a) as an addition to campylobacter media to counteract the toxic effect of oxygen (Hoffman et al., 1979b). Many selective media contain some or all of these compounds but concentrations vary. A few media contain both FBP supplement *and* blood (e.g. Gilchrist et al., 1981; Weber et al., 1987; Stern et al., 1992) but in most cases media contain either blood or FBP. Alternatives to blood or FBP include haematin (Razi and Park, 1979; Wesley et al., 1983; Karmali et al., 1986) and charcoal (Bolton et al., 1984; Karmali et al., 1986). Lignite-derived humic acids plus 0.05% $FeSO_4 \cdot 7H_2O$ have been proposed as an alternative system (Weinrich et al., 1990). The mode of action of these supplements is not clear, but they may help to neutralise hydrogen peroxide, singlet oxygen and/or superoxide ions.

4. Storage of campylobacter plating media

Hoffman et al. (1979a) found that growth of campylobacters was substantially reduced when plates of nutrient medium were stored in the presence of light and, in particular, air. Similar results were obtained by Bolton et al. (1984) and Juven and Rosenthal (1985). The incorporation of aerotolerant supplements such as FBP or blood into campylobacter media reduces this effect but does not completely eliminate it (Fricker, 1985; Weinrich et al., 1990). Preston agar stored aerobically at room temperature inhibits the growth of *C. lari* to a greater extent than *C. jejuni* or *C. coli* (Fricker, 1985). Loss of moisture is probably also a factor (Buck and Kelly, 1981; Fraser et al., 1992). Campylobacter plates prepared in the laboratory should therefore either be used immediately or after storage in the dark anaerobically at room temperature, or aerobically in the refrigerator with precautions to prevent dehydration. A maximum storage time of 5 days for laboratory prepared plates is probably advisable.

5. pH

The pH of many of the isolation media is not specified, but presumably approximates to that of the basal medium used, normally near neutrality. Some liquid enrichment media have a pH ca. 8.0 (Wesley et al., 1983) although Park et al. (1983) found that their medium functioned best at pH 7.0. High pH media could be used to counteract acid production by competitive flora. The ability of competitive flora to acidify the enrichment media may depend on the food under examination (Humphrey, 1986b).

6. Choice of antibiotics

Antibiotics in selective media developed for campylobacters were chosen on the basis of those to which test strains were resistant and those most effective in

inhibiting competitive flora. Table 4 lists the results of various published studies on the resistance of campylobacters to antibiotics. Neither Butzler (Dekeyser et al., 1972; Butzler et al., 1973; Lauwers et al., 1978), Skirrow (Skirrow, 1977; Butzler and Skirrow, 1979) nor Blaser (Blaser et al., 1979) published the full rationale for the development of their media. Probably these authors used data from Plastridge et al. (1964), Butzler et al. (1974) and Vanhoof et al. (1978). Later workers will have had access to other studies listed in Table 4. Plastridge et al. examined 57 strains of 'related vibrio' bacteria that probably belonged to the *C. jejuni / C. coli* group, finding them resistant to bacitracin, novobiocin and polymyxin B. Butzler et al. (1974) found over 90% of 114 strains of related vibrios resistant to cephalothin and all were resistant to vancomycin and rifampicin. With few exceptions (Ng et al., 1985 and presumably Plastridge et al., 1964) the strains tested had been isolated using antibiotic-containing selective media and so the possibility of missing strains sensitive to the antibiotic used in the selective media will have been perpetuated.

7. Antibiotic activity

Polymyxin is generally active only against Gram-negative bacteria, and *Proteus* spp. are sometimes resistant. Trimethoprim usually inhibits *Proteus* spp. as well as other Gram-negative bacteria. Colistin is closely related to polymyxin, has a similar spectrum of activity and is used in some media instead. Vancomycin and rifampicin are both used, but not usually together. They are effective against Gram-positive bacteria. Rifampicin is also active against Gram-negative organisms. Butzler's media have generally incorporated bacitracin instead of vancomycin. The cephalosporins (cephalothin, cefaperazone) have a wide spectrum of activity against Gram-positive bacteria. Cefoperazone is now usually preferred because some campylobacters, especially *C. coli*, are sensitive to cephalothin (Brooks et al., 1986; Ng et al., 1985, 1988; Burnens and Nicolet, 1992). Cycloheximide (actidione), amphotericin B or nystatin are present in many media to inhibit yeasts and moulds.

8. Development of plating media (Table 3)

Bolton and co-workers gave the most detailed rationale for their formulation of Preston and CCD (charcoal, cefazolin (later replaced by cefoperazone) deoxycholate) media. When developing Preston medium, Bolton and Robertson (1982) carried out a survey of the MIC's of 104 strains of *Campylobacter* spp. and a variety of competitive organisms against four antibiotics (polymyxin, rifampicin, vancomycin and trimethoprim). They chose polymyxin because of its activity against Gram-negative bacteria (except for *Proteus* spp., which were suppressed by the trimethoprim). Because rifampicin showed a wide spectrum of activity against Gram-positive and Gram-negative bacteria it was chosen in preference to vancomycin, which has limited activity against Gram-negative organisms. Ampho-

Table 4a
Campylobacter antibiotic resistance profiles obtained by various authors (mic, mg/l required to inhibit)

Authors	Plastridge et al., 1964		Vanhoof et al., 1978	Walder et al., 1979	Karmali et al., 1980	
Species of *Campylobacter*	*jejuni* (57) * (grp II)	*coli* (61) (grps III & IV)	*jejuni* (95)	*jejuni* (100)	*jejuni* (60)	*fetus* (12)
ampicillin			≤ 0.195–50	2.5–40		
cloxacillin			> 100			
moxalactam						
cefazolin					128– ≥ 512	8–32
cefotaxime				5–40	2–32	4–16
cephalothin			6.25– > 100	> 160	128– ≥ 512	16–32
cefoxitin			6.25– > 100		32– ≥ 512	32–64
cephalexin			6.25– > 100	40–160	16– ≥ 512	32–128
cefuroxime			6.25– > 100	40–160		
cefoperazone						
cefamandole					128 ≥ 512	16–64
chloramphenicol			0.39–6.25	3.1–12.5		
polymyxin-B	1024	4–16	1.56–100			
colistin			6.25– > 100			
bacitracin	128	64				
trimethoprim			50–200	> 160		
rifampicin			6.25– > 100			
vancomycin			50– > 100			
novobiocin	128	256–512				
sulphamethoxazole			1.95–250	5–80		
METHOD	Nutrient agar + 5% ox blood		Mueller-Hinton agar (for trimethoprim & sulphamethoxazole plus 5% lysed horse blood)	Mueller-Hinton agar + 5% human blood	DST + 5% lysed horse blood	

Key: DST, diagnostic sensitivity test agar, IST, isosensitivity test agar; * number of strains tested; ** iu per ml.

Table 4b

Authors	Ahonkai et al., 1981	Karmali et al., 1981		Michel et al., 1983	Ng et al., 1985	
Species of *Campylobacter*	*jejuni* (36) *	*jejuni*		*jejuni* (103) *	*jejuni* (6) *	*coli* (24) *
ampicillin	2–8	1–256	(157) *	⩽ 0.125–64		
cloxacillin		64– ⩾ 512	(105)			
moxalactam	4–32	2–128	(95)			
cefazolin		128– ⩾ 512	(60)	⩽ 512		
cefotaxime	2–4	1–32	(106)			
cephalothin		64– ⩾ 512	(108)		128– ⩾ 256	32–256
cefoxitin		32– ⩾ 512	(107)			
cephalexin		16– ⩾ 512	(104)			
cefuroxime						
cefoperazone	128					
cefamandole		128– ⩾ 512	(107)			
chloramphenicol	2–8	⩽ 0.5–8	(142)	⩽ 0.5– ⩾ 64		
polymyxin-B		1–32	(97)	1–32	2–8 (16-64iu)	0.5–8 (4.6iu)
colistin				0.5–32	4–32	2–32
bacitracin		⩾ 512	(139)		> 512	> 512
trimethoprim		256– ⩾ 512	(56)	256– ⩾ 512	256	128–256
rifampicin	32–128	8–128	(91)		32–128	2–128
vancomycin	128	128– ⩽ 512	(77)		> 128	> 128
novobiocin		64– ⩽ 512	(88)		32–64	1–128
sulphamethoxazole		8–256	(55)	8–512		
METHOD	Mueller-Hinton broth + laked blood	DST + 5% lysed horse blood		IST + 7% lysed human blood	Muller-Hinton agar	

See Table 4a for key.

Table 4c

Authors	Gebhart et al., 1985		Ng et al., 1988		Kiehlbauch et al., 1992		Patton et al., 1989
Species of *Campylobacter*	*coli* (10) *	*hyointestinalis* (10) *	*jejuni* (60) *	*coli* (101) *	*cryoaerophila* (12) *	*butzleri* (64) *	*upsaliensis* (12)*
ampicillin	4.8	0.25–0.5			8– > 32	4– > 32	1–32
cloxacillin							
moxalactam							1
cefazolin	128	32–64					
cefotaxime					8– > 64	4– > 64	0.5–4
cephalothin	128	16–64	64–128	32– > 256	> 32	32– > 32	1–32
cefoxitin	128	16–64					1–8
cephalexin							
cefuroxime					16– > 32	8– > 32	
cefoperazone					16– > 32	16– > 32	
cefamandole							1–16
chloramphenicol	2–16	2–16			4– > 32	8– > 32	2–8
polymyxin-B	64– ⩾ 128	64– ⩾ 128	< 1–8	< 1–8	⩽ 2.5 **	⩽ 2.5 **	
colistin	⩾ 128 **	⩾ 128 **	1–16	< 1–16			
bacitracin	⩾ 128 **	⩾ 128 **	256– > 512	256–512			
trimethoprim	⩾ 128	64– ⩾ 128	256– > 256	256– > 256	> 50	> 50	
rifampicin	32–64	32–64	4–128	16– > 128	⩽ 10 **	⩽ 10 **	
vancomycin	⩾ 128	⩾ 128	128– > 128	128– > 128	> 10	> 10	32
novobiocin	16–32	32–64	16–128	8–128			
sulphamethoxazole							
METHOD	Mueller-Hinton agar		Mueller-Hinton agar		Mueller-Hinton broth + 5% lysed horse blood		

See Table 4a for key.

tericin B was added to inhibit yeasts. The level of polymyxin was set at 5000 iu per litre on the basis of Vanhoof's results, which showed that some campylobacters were sensitive to 10000iu per litre. When developing their CCD medium Bolton and co-workers determined that campylobacters grow best on solidified nutrient broth no. 2, after comparison with other media such as Columbia blood agar base, veal or brain heart infusion and diagnostic sensitivity test agar. They then carried out a systematic survey of alternatives to blood for neutralising oxygen toxicity by comparison with the growth of campylobacter obtained on nutrient agar plus 5% lysed horse blood (Bolton and Coates, 1983; Bolton et al., 1984a). A combination of 0.4% charcoal, 0.25% ferrous suiphate and 0.25% sodium pyruvate was best. A further study (Bolton et al., 1984b) surveyed the effect of 11 dyes, 17 chemical compounds and 14 chemotherapeutic agents on one strain each of *C. jejuni* biotype 1, *C. jejuni* biotype 2, *C. coli* and a NARTC (nalidixic acid resistant thermophilic campylobacter – *C. lari*) and a selection of Gram-positive and Gram-negative competitive bacteria. No details were given of either the competitive test strains or the inhibitors examined, but deoxycholate and cefazolin were chosen as the most effective inhibitory agents. Casein hydrolysate was found necessary to stimulate the growth of environmental NARTC (*C. lari*) strains. CCDA, apart from Karmali's medium, is the only widely used plating medium not using blood. This is useful, particularly for laboratories specialising in food microbiology in which the use of blood in media is uncommon. Blood is expensive, has a short shelf life and is easily contaminated. Later Hutchinson and Bolton (1984) replaced cefazolin (10 mg/l) by cefoperazone (32 mg/l). This allowed fewer contaminants to grow, and permitted the modified medium (mCCDA) to be used at 37°C. However, amphotericin B was needed to prevent overgrowth by yeasts able to grow at 37°C but not at 42°C.

No rationale is provided by Blaser et al. (1979) for their 'Campy-BAP' medium, which has a similar formulation to Skirrow (1977) agar, but with added cephalothin, as used by Lauwers et al. (1978).

In 1983, Butzler and co-workers produced a second selective plating medium called Butzler medium Virion (Goossens et al., 1983). This was said to be superior to a slightly modified version of their 1978 medium (Lauwers et al., 1978), 'Butzler medium Oxoid', which had a different basal medium, less blood and cephazolin instead of cephalothin. Butzler medium Virion substituted cephoperazone for cefazolin, rifampicin for vancomycin, colistin for polymyxin and trimethroprim and amphotericin B for cycloheximide. The Virion medium was said to suppress competing flora more effectively, especially pseudomonads and Enterobacteriaceae. Campylobacter colonies were easier to recognise. Incubation temperature was reduced from 42°C to 37°C (Butzler et al., 1983). Goossens et al. (1986) modified the Virion medium, omitting the colistin and doubling the concentration of cefoperazone in order to detect colistin-sensitive campylobacters, including some strains of *C. coli*, reported by Ng et al. (1985) to be sensitive to colistin and polymyxin. Goossens and co-workers found no significant improvement over their Butzler Virion agar. However, they recommended its use in preference to the earlier medium, presumably because they felt it would be able to isolate colistin-

and polymyxin-sensitive campylobacters. Burnens and Nicolet (1992) were able to isolate *C. upsaliensis* after changing to the medium of Goossens et al. (1986).

The medium of Karmali et al. (1986) is a variation of modified CCD medium (Hutchinson and Bolton, 1984) using haematin rather than ferrous sulphate, vancomycin instead of deoxycholate and cycloheximide instead of amphotericin B. Vancomycin at 20 mg per litre rather than the more common 10 mg per litre was chosen for better suppression of Gram-positive competitors (*Bacillus* spp. and enterococci). Cycloheximide was chosen because of superior yeast suppression. In agreement with Hutchinson and Bolton (1984) cefoperazone was found superior to cephalothin. The most numerous contaminants were found to be Enterobacteriaceae which are resistant to cefoperazone when present in high numbers, especially *Klebsiella oxytoca*. The rationale for the modification to the oxygen quenching system was not stated.

The semi-solid medium of Goossens et al. (1989) relies upon the ability of campylobacters to swarm, analogous to the system used in semi-solid Rappaport Vassiliadis media for salmonellas (Busse, 1993). The use of a semi-solid medium, where growth and swarming is mostly below the surface apparently dispenses with the need for blood, charcoal or other anti-oxygen system other than a microaerobic atmosphere. The medium has to be stored in the dark at 4°C, and prepared twice weekly. Cefoperazone (30 mg/l) and a high level of trimethoprim (50 mg/l) are used as selective agents. Three strains of *C. jejuni* and 7 strains of *C. coli* with MIC's to cefoperazone < 100 μg/ml (screened in a previous study of 200 inhibitors) were tested for their ability to swarm in the new medium; all gave satisfactory results. Eleven different campylobacter strains were tested for their ability to initiate swarming in the medium. A range of 1–15 cells was necessary, with a mean of 6.7. The medium functioned very well by comparison with other plating media. Neat faecal samples were inoculated using a loop at the edge of 50 mm diameter Petri dishes of the medium. Incubation was at 42°C for 42 h in a microaerobic atmosphere or a candle jar. Positive campylobacter cultures were recognised by the characteristic swarming growth. Presumably isolation required subculture to selective or non-selective plates to obtain a pure growth. Polymyxin or other Gram-positive inhibitors, as well as anti-fungal antibiotics were not necessary because competitor bacteria did not swarm. The medium had a number of advantages: cheapness because of the small volumes needed, no necessity for blood, only two antibiotics in the formula, easy interpretation of swarming, low incidence of contaminants, and the ability to fit large numbers of 50 mm plates into gas jars. A disadvantage would be the unsuitability of semi-solid agar for quantitative estimates of campylobacters, unless MPN techniques were used.

Recently Aspinall et al. (1993) have developed a modification of mCCD agar designed to isolate *C. upsaliensis* as well as the other thermophilic campylobacters at 37°C. Of 51 strains of *C. upsaliensis* tested, 47 were resistant to 8 mg per litre of cefoperazone in CCD agar base and all were resistant to 64 mg per litre of teicoplanin. The medium contains 8 mg/l cefoperazone and 4 mg/l teicoplanin, replacing 32 mg/l cefoperazone in mCCD agar (Table 3). Teicoplanin has an antimicrobial spectrum similar to that of vancomycin, active mainly against Gram-

positive bacteria. By comparison with mCDD agar the final formulation isolated equivalent numbers of *Campylobacter* spp. other than *C. upsaliensis* from faeces and was superior to mCCD agar for *C. upsaliensis*, with slightly more growth of competitors. These results were confirmed using faeces artificially inoculated with *C. upsaliensis*.

9. Membrane filter method of Steele and McDermott (1984)

This method is a simplification of the filtration method of Dekeyser et al. (1972). It employs 47 mm 0.45 μm cellulose triacetate membrane filters, placed centrally on a 90 mm 6% sheep blood agar plate. Ten to twelve drops of a 1 in 10 suspension of faeces in physiological saline solution are placed onto the membrane filter using a Pasteur pipette and taking care not to let the drops spill over the edge of the membrane. The membrane is removed after 30 min, by which time the fluid (and presumably sufficient campylobacters) will have passed through. The plates are then incubated micro-aerobically at 41°C ± 1°C for 3–5 days examining daily. Cellulose nitrate filters were unsatisfactory and 0.8 μm pore size gave unacceptable levels of contamination; 0.65 μm filters were not tested.

Comparison of the filter method with a selective medium containing sheep blood, FBP supplement, trimethoprim, vancomycin and colistin gave 45 positive with the selective medium and 50 with the filter method out of a total of 56 positive samples from the 1000 tested. Six strains of *C. jejuni* were not isolated by the filter method and 11 *Campylobacter* spp. were not isolated with the selective medium. Six of these were *C. jejuni*. None of the non-*C. jejuni* strains grew in the selective agar because of the colistin present (they were also sensitive to polymyxin). These authors found that the filter method usually gave either campylobacter colonies or no colonies at all. However, only about 10% of campylobacters in the faecal suspension actually passed through the filter. This method or a similar one (e.g. using 0.65 μm filters) has been used by various workers, often in parallel with selective agar media or after selective enrichment (Megraud and Gavinet, 1987; Goossens et al., 1990b; De Boer and Reitsma, 1991; Moreno et al., 1993; Scotter et al., 1993). Shanker et al. (1991), working with 0.45 μm cellulose acetate or mixed cellulose ester filters and artificially contaminated faeces, found that 10^4/g of most strains of campylobacter could be detected. In their procedure the filter was removed 10 minutes after applying the faecal suspension.

10. Enrichment methods for isolation from food

The Preston and mCCD media of Bolton and co-workers have been utilised as enrichment media using the same inhibitors, and incubation at 37 or 42°C. Liquid Preston medium contains 0.25 g/l FBP in addition to 5% horse blood (Bolton et al., 1982; Baird et al. 1987). Both media have been found useful for isolating campylobacters from samples in which campylobacter numbers are comparatively

low (e.g. faeces of normal animals, water and drain swabs – Bolton et al., 1982, 1983).

Park et al. (1981) developed an enrichment broth based on Brucella broth, containing vancomycin, trimethroprim and polymyxin B (VTP). It was later modified (Park et al., 1983) by adding FBP supplement and lysed horse blood, reducing the amount of the polymyxin B and increasing that of vancomycin and trimethoprim (VTP-FBP) – see Table 3. The media were used to examine raw chickens. Carcasses were rinsed with nutrient broth, which was then filtered through cheesecloth and centrifuged. The sediment was resuspended in Brucella broth and then either plated directly onto selective agar or added to the enrichment broth. Incubation was carried out at 37°C under microaerobic conditions. VPT broth was found to detect 0.2 campylobacters per g of sediment in the presence of 10^4–10^6 competitors per g (Park et al., 1981). Enrichment showed 62% of chickens to be positive for campylobacters, compared with 32% by direct plating. VPT-FBP broth functioned better at 37°C than 42°C and 48 h incubation was better than 24 h or 72 h. pH 7.0 was optimal.

Christopher et al. (1982) used a liquid version of Campy-BAP agar (see Table 3) with added pyruvate. This medium contained cephalothin and amphotericin B in addition to vancomycin, polymyxin and trimethroprim. Ehlers et al. (1982) modified this medium by adding FBP. The broth was incubated at 42°C in microaerobic atmosphere. The limit of detection in cheese artificially contaminated with *C. jejuni* and using an MPN method was about 0.3 campylobacters per g. Acuff et al. (1982) published a similar modification containing FBP, but without blood. Doyle and Roman (1982) developed a broth similar to that of Park and co-workers, but with a very high level of polymyxin (20,000 iu per litre), cycloheximide and an oxygen quenching system of 7% lysed horse blood, as well as added succinate and cysteine HCl. Incubation was microaerobic at 42°C, for 16–18 h. The medium was evaluated using a variety of foods artificially inoculated with 46 strains of *C. jejuni* 2 of *C. coli* and 2 of *C. lari*. 10 or 25 g of food were homogenised directly in 90 or 100 ml of enrichment broth. Campy-BAP agar was used as the plating medium. All strains in raw hamburger and raw milk were detected at 1–4 cells per g and most at 0.1 to 0.4 cells per g. The broth was less effective for chicken, probably due to the numbers and types of competitors.

Wesley et al. (1983) used a medium (alkaline tryptone broth) containing rifampicin, polymyxin at 20,000 iu per litre and 6.25 mg/l of cefsulodin. The levels of polymyxin and cefsulodin chosen were necessary to inhibit *Pseudomonas aeruginosa* contaminants. FBP and haematin were included as oxygen quenchers and the optimum pH level was 8.0. 100 ml of broth was inoculated with 10 ml of carcass rinse fluid. Incubation was microaerobic at 42°C for 48 h. Comparison of this medium with the medium and sampling method of Park et al. (1981) for isolation of campylobacters from naturally contaminated chicken gave a much higher isolation rate (25 versus 6 out of 50 chickens using the Wesley or Park medium respectively).

Lovett et al. (1983) modified the medium of Park et al. (1981). They added FBP and as a result of testing growth of *C. jejuni* strains and competitors, reduced the

level of polymyxin and increased the levels of the other antibiotics (Table 3). The 8,000 iu per litre of polymyxin used in the Park formulation was found to be inhibitory to many strains of *C. jejuni*.

11. Effect of damage

Ray and Johnson (1984a,b) observed that viability of freeze-injured *C. jejuni* was reduced when cells were incubated in selective broth or (to a lesser extent) on selective agar at 42°C. They used the liquid medium of Acuff et al. (1982) and Campy-BAP agar. The basal media without antibiotics were also inhibitory, an effect attributed partly to the high incubation temperature. Blood reduced the toxicity, as also did succinate and cysteine. Polymyxin was toxic to the injured cells but the other antibiotics were not. As a result of these studies a medium was proposed using a modification of the broth of Ehlers et al. (1982), which contained succinate and cysteine but no FBP supplement. Incubation was at 37°C for the first 6 h to resuscitate injured cells before adding the polymyxin and raising the incubation temperature to 42°C.

Humphrey and Cruickshank (1985) investigated the effect of a variety of inhibitors used in selective media on 6 serotypes of *C. jejuni* uninjured or injured by freezing, heating or EDTA. Bacitracin, trimethoprim, cefoperazone, colistin, novobiocin, vancomycin and deoxycholate were tested in blood agar, all at levels used in selective media. The growth rate and colony size of undamaged strains were reduced by rifampicin. Injured cells were more affected. Deoxycholate was toxic for some but not all injured cells. The toxic effect of rifampicin and deoxycholate in complete medium was confirmed for one strain when freeze-damaged. Counts on Preston medium were reduced compared to Skirrow, Campy-BAP or blood agar for one of the 6 strains. Further work (Humphrey 1986a,b) confirmed the observations of Ray and Johnson (1984) that damaged *C. jejuni* recovered better in media at 37°C than 42 or 43°C, even in the absence of inhibitors. An enrichment medium and method was subsequently proposed which had nutrient broth as the base. To this was added 5% lysed horse blood and FBP. Antibiotics were omitted for the initial period of incubation. Food samples were incubated in the basal broth for 2 h at 37°C before addition of the antibiotics and incubation was continued at 43°C for up to 48 h. The antibiotics were trimethoprim, cefoperazone, colistin, amphotericin B and either vancomycin or rifampicin (see Table 3). Some inhibition of *C. jejuni* was observed even after the 2 h 37°C resuscitation period. Vancomycin was less inhibitory than rifampicin. No information was supplied about the incubation atmosphere. The plating medium used following enrichment had a similar formulation, but substituted rifampicin for vancomycin in order to suppress competing flora. In a later paper Humphrey (1989) suggested that pre-enrichment at 37°C should be continued for 4 h and that addition of all antibiotics should be delayed until the 4 h pre-enrichment had been completed. The rifampicin-containing enrichment medium was recommended in parallel with a similar plating medium (Humphrey 1986b; Table 3). Pre-enrichment

at 37°C for 4 h was recommended for all types of sample, even those heavily contaminated, such as sewage and chicken skin, although for these, selective medium rather than the basal enrichment broth, was recommended. Later the broth was modified to contain polymyxin instead of colistin, because of difficulty in obtaining colistin (De Boer and Humphrey, 1991; Humphrey, personal communication).

A similar enrichment medium for use with food was suggested by Bolton (Table 3; personal communication; Anon., undated). It has a rich basal medium to aid resuscitation of sublethally damaged campylobacters. Similarly to Humphrey's Exeter enrichment medium, preliminary incubation of the medium complete with antibiotics, for 4 h at 37°C was recommended to aid resuscitation of injured organisms, followed by 42°C for 14–48 h. The combination of some of the FBP ingredients with lysed horse blood and haemin, together with a small headspace in the culture vessels was designed to avoid the need for a microaerobic atmosphere. Sodium carbonate was added, presumably to prevent acidification and/or provide a source of carbon dioxide. The broth is used in a 1:4 (w/v) ratio of food to medium, with a 1.5 cm headspace in a screw-capped bottle (presumably with the cap tightened). It can also be used to enrich faeces (1 ml of 10% suspension added to 5 ml broth).

Another enrichment method employing similar principles was published in abstract only by Park and Sanders (1991) (see Table 3 for formulation). No rationale was supplied for the choice of antibiotics. The method involved incubation of the broth containing vancomycin and trimethoprim for 4 h at 31–32°C, followed by addition of cefoperazone and cycloheximide. Incubation temperature was raised to 37°C for 2 h and then 42°C for 40–42 h. All incubation was microaerobic; static at 30–32° and 37°, and shaking at 42°C.

Yet another enrichment medium, a variation of Park and Sanders broth, also employing a period of incubation at reduced temperature, and delayed addition of antibiotics was devised by Hunt and Radle (cit. Stern and Line, 1992; Hunt, 1992). This medium contains 0.25 g/l FBP and 5% lysed horse blood, cefoperazone, trimethoprim, vancomycin, and amphotericin B or cycloheximide (see Table 3).

Pre-enrichment is carried out in the medium containing all the antibiotics (except for 15 mg/l cefoperazone of the final total of 30 mg/l) at 32°C in a microaerobic atmosphere in flasks sealed in plastic bags and shaken in a water bath. After 3 h the remainder of the cefoperazone is added and the temperature of the water bath raised to 37°C. After a further 2 h at 37°C, the bath temperature is raised to 42°C.

The FDA Bacteriological Analytical Manual (1992) recommends use of Hunt and Radle broth for most types of food, modified Exeter broth for water and environmental samples and Hunt and Radle broth and modified Preston agar for dairy products. Frozen products or products stored chilled for 10 days or more are pre-enriched according to the Hunt and Radle protocol or the Humphrey (1989) protocol. Foods not chill-stored are examined by the Hunt and Radle protocol, but adding all antibiotics at the beginning of incubation. Shaking water baths are recommended for incubation, with continuous gas flow (except during incubation

at 30°C if it is static). Plating is on mCCDA and Campy-Cefex directly and after enrichment. In general 25 g food is added to 100 ml of enrichment broth. Liquid products such as milk and ice cream are centrifuged. Chickens are rinsed, the rinse fluid centrifuged and the pellet added to the enrichment broth.

12. Atmosphere during incubation of enrichment media

No systematic investigation of the effect of atmosphere during incubation of liquid media has been carried out. Some earlier workers used static incubation in normal air in screw-capped bottles, sometimes with a small airspace, sometimes with the airspace undefined (Bolton et al., 1982b; Fricker et al., 1983; Martin et al., 1983; Waterman et al., 1984). Others used static incubation in bottles or tubes in a microaerobic atmosphere (Acuff et al., 1982; Ehlers et al., 1982; Megraud, 1987; De Boer and Reitsma, 1991). Doyle and Roman (1984) used 250 ml conical flasks possessing side-arms and replaced the atmosphere with the microaerobic gas mixture. They were evacuated and filled three times. Park et al. (1981, 1983) and Lovett et al. (1983) bubbled microaerobic gas mixture through their enrichment broths. Park and Sanders (1991) used static incubation of plastic storage bags flushed with microaerobic atmosphere. The report of Ribeiro and Price (1984) who used Preston broth containing FBP supplement (George et al., 1978) to isolate campylobacters from water indicated that a microaerobic atmosphere during incubation gave a higher isolation rate. Wesley et al. (1983) reported that better results were obtained with a high surface area of broth in a flask compared to a lower surface area in tubes, in both cases using a microaerobic atmosphere. Comparison of Doyle and Roman's evacuation-replacement procedure with a continuous gas flow procedure using Doyle and Roman's enrichment broth for examining inoculated hamburgers indicated that the continuous gas flow gave counts 1–2 log cycles higher for 6 out of 8 strains of *C. jejuni* for both tests (Heisick et al., 1984). There appears to be no published justification for the FDA recommendation of continuous gas flow with agitation (Hunt, 1992).

13. Comparisons of media

The most important comparisons will be discussed in chronological order. Of course the earlier comparisons could not examine media only later developed, so that the most recent comparisons are likely to be of greatest interest. Comparisons published to support the development of a new medium have only been cited where several other media have been compared. There are few comparisons of media for isolation from food, so this review will also include studies for isolation from faeces and water.

Morris et al. (1982) compared plating media for isolating *C. jejuni* from (1) dog and cat faeces and (2) human diarrhoeal faeces. In the first study BU40 (a modification of Butzler's medium (Butzler and Skirrow, 1979) with 40,000 rather

than 10,000 iu per litre colistin (Patton et al., 1981), was compared with Skirrow and Butzler medium. Eighty-seven out of 347 samples were positive for *C. jejuni*. BU40 gave significantly higher numbers of positive samples than Butzler's or Skirrow's media but Butzler and Skirrow media were not significantly different from one another. No two media detected all the positive samples but Skirrow + BU40 detected 98% of them. Detection of *C. jejuni* on Skirrow medium was sometimes hampered by swarming *Proteus* spp.. Coliforms were sometimes a problem on Skirrow and Butzler media, but not on BU40, and *Providencia* spp. were inhibited only by Skirrow's medium. In the second study, Campy-BAP, Skirrow, Butzler and BU40 agars were compared. No significant differences were found between these four plating media. A greater number of contaminants was found on Skirrow's medium. The difference between the results in the two studies may have been because the human faeces examined in the second study were from subjects with diarrhoea and hence campylobacters were present in high numbers. In both surveys it was necessary to check the plates at 24, 48 and 72 h in order to detect all positive samples.

Bolton et al. (1983) compared Skirrow, Butzler (with cephazolin), Campy-BAP, Blaser agar (a modification of Campy-BAP, with 5% horse blood and blood agar base No. 2) and Preston media. Pure cultures of two strains each of *C. jejuni*, *C. coli* and NARTC (*C. lari*) were tested. All the media reduced (i.e. up to 1 log cycle) the counts of pure cultures of at least some strains compared with a non-selective (5% lysed blood) agar. Butzler medium was judged unsuitable for cultivating *C. coli* and *C. lari* (reduction of over 3 log cycles). Examination of campylobacter-positive human faeces and faecal samples from sheep, pigs, cattle and chickens, plus abattoir drain swabs indicated that no medium detected all positive samples. The order of efficiency of isolation was Preston (84%), Blaser (76%), Skirrow (74%), Campy-BAP (69%), Butzler (68%). In terms of rate of contamination with unwanted organisms, Preston agar had fewest followed by Butzler, Skirrow, Blaser and Campy-BAP. The most frequent contaminants were coliforms, *Pseudomonas* spp. and streptococci. Growth of yeasts occurred on Skirrow, Butzler and Preston media. Plates read after 48 h at 42°C gave consistently higher isolation rates than plates read at 24 h. Isolation rates were not significantly higher after 72 h, because of overgrowth by contaminants. The use of Preston enrichment broth improved isolation rates from samples with comparatively low numbers of campylobacters – e.g. sheep and cattle rectal swabs and abattoir drain swabs. The observation that Blaser agar gave better results than Campy-BAP when the two media were identical except for differing basal media and blood content is of interest. Campy-BAP was also the only medium supplied ready-made and this might have had an effect. Concerning poor growth of *C. lari* and *C. coli* Bolton et al. commented that they had found that Butzler's medium based on Columbia agar and 5% horse blood, instead of blood agar base no. 2 and 5% horse blood, recovered campylobacters much more effectively from avian and porcine samples, where *C. lari* and *C. coli* respectively predominate. The later Butzler media (Oxoid and Virion) used Columbia agar base.

Fricker et al. (1983) compared Preston, Skirrow, Butzler (with cephazolin) and

Blaser (as used by Bolton et al. 1983) agars, for plating seagull faeces directly or after enrichment in Preston or Roman and Doyle's enrichment medium. Enrichment greatly increased the isolation rate. The highest rate of isolation occurred using Preston enrichment onto Preston agar. There was little difference in the performance of the four selective agar media, except that *C. lari* and *C. coli* strains grew very poorly on Butzler medium, confirming the observations of Bolton et al. (1983).

Merino et al. (1986) tested seven plating media for the examination of 263 samples of diarrhoeal faeces from humans. Butzler (with cephazolin, basal medium not clear), Blaser, Skirrow, Preston, mCCDA, Butzler Virion and Preston with 2 mg/l amphotericin B. All the media isolated campylobacter at similar frequency, but mCDDA was preferred because it gave the lowest number of plates with contaminating flora. Incubation at 43°C for 24 h was considered optimal. Further incubation until 48 h resulted in overgrowth of contaminants.

Rosef et al. (1987) compared methods for isolating campylobacters from naturally contaminated water. In the first part of their study they filtered the water through 0.45 mm and 0.22 mm membranes and compared recovery when the filters were placed in Preston, Rosef or Rosef + FBP supplement enrichment broth (42°–43°C, incubated in microaerobic atmosphere for 24–48 h), with the recovery obtained when the filters were laid face up on Skirrow or CAK agar. Of forty positive samples, 39 were detected without enrichment (using CAK and Skirrow agar), 27 were detected by use of Skirrow agar alone and 35 using CAK agar alone. All positive samples were detected using 0.45 μm filters and only one using the 0.22 μm filter. The best enrichment medium was Preston, followed by Rosef + FBP. In the second part of the study the water was passed through various combinations of 0.65, 0.45 and 0.22 μm filters which were then placed face up on CAK agar. The 0.45 μm filter used alone gave the highest isolation rate followed by the 0.65 μm filter used alone. In the third part Preston, CAK and Skirrow agars were compared, inoculating with 0.45 μm filters, face up. Preston detected the greatest number of positive samples, followed by CAK, with Skirrow detecting substantially fewer. All three media needed to be incubated for 48 h in order to detect the maximum number of positive samples. No bias was observed in proportions of *C. jejuni*, *C. coli* and *C. lari* isolated on the three plating media. In a similar study, using 0.45 μm filters, Brennhovd and Kapperud (1991) compared enrichment in Preston broth followed by plating on Preston agar or CAK agar, with direct plating of the filters onto Preston or CAK agar. Preston agar gave higher numbers of positive samples than CAK agar by direct plating. Direct plating on Preston agar, together with enrichment in Preston broth with subculture to Preston agar, detected 93% of positive samples. It was necessary to examine plates at 48 h as well as 24 h incubation.

Gun-Munro et al. (1987) compared counts of pure cultures of 70 strains of *C. jejuni* on Skirrow, Butzler, Blaser-Wang, Preston, mCCDA and Karmali media. All except Skirrow, mCCDA and Karmali gave slightly but significantly lower mean counts than non-selective blood agar. 'Simulated faeces' samples were prepared by inoculating each of seventy different faeces samples, previously found to be

negative for campylobacters, with one of the seventy pure cultures of *C. jejuni* at a level of 10^5 per ml faeces. The six media were scored in terms of numbers of simulated faeces samples found positive, as well as their ability to inhibit the inoculated faecal flora. The percent positive samples detected at 48 h for each medium was as follows: Blaser-Wang 41%; Butzler 86%; Preston 91%; Skirrow 96%; Karmali 97%; mCCDA 99%. Karmali and mCCDA suppressed the competitive flora more effectively. Karmali was chosen rather than mCCDA to compare with Skirrow medium against almost 2,556 human faecal specimens and 224 animal and bird specimens. This was because the authors found campylobacter colonies easier to recognise on Karmali than on mCCDA. There was little to choose between Skirrow and Karmali medium in terms of productivity, with Karmali isolating a few more strains from human faeces in transport media, and from chicken faeces. Skirrow medium gave better results on faeces from other animals. However, Karmali medium was much more selective and therefore easier to use. For instance at 48 h the faecal flora was suppressed by 75% or more with 57% of the samples on Karmali, compared with only 32% suppression on the Skirrow medium.

The study by Albert et al. (1992) on human diarrhoeal stools compared mCCDA incubated at 42°C with the membrane filter method of Steele and McDermott (1984) but using a 0.65 μm pore membrane filter and incubating at 37°C. The filter method isolated many more campylobacters, mostly uncharacterised *Campylobacter* spp., *C. upsaliensis* and *C. jejuni* subsp. *doylei*, but also significantly more *C. jejuni*. Multiple strains of campylobacter were often isolated from one faecal sample using the filter method. The differing performance of the two methods could have been due to the different incubation temperatures and/or the antibiotics in the selective medium. Megraud and Gavinet (1987) compared isolation rates from human faeces after direct plating on Butzler medium Virion at 42°C for 2 days with membrane filtration using a 0.65 μm cellulose acetate filter (incubating at 37°C) or with membrane filtration after enrichment in Preston broth (all incubated at 37°C and plates incubated for 5–7 days). The enrichment filtration method was most sensitive and direct plating least sensitive. Better results were obtained with Millipore membranes (cellulose nitrate and acetate) than Sartorius membranes (cellulose nitrate).

Moreno et al. (1993) examined faecal samples from various domestic pets and compared with direct plating on mCCDA two methods of filtration through a 0.65 μm pore (Millipore) filters onto 5% horse blood agar. Incubation was at 37°C for periods up to 8 days. A filtration procedure was superior to direct plating for isolating campylobacters (particularly *C. upsaliensis*) from this type of specimen. A method that involved initial low speed centrifugation of the faecal suspension (10%) in order to prevent clogging of the filter, followed by filtration through a 0.65 μm filter and high speed centrifugation in order to concentrate the organisms, was superior to the Steele and McDermott (1984) method.

14. Isolation from foods

Beuchat (1985) compared Campy-BAP, CCDA (unmodified – Table 3 – Bolton and Coates (1983)) and Butzler agar (as in Table 3: with cephalothin), plus five enrichment media (by MPN) for the isolation of campylobacters from chill-stored chicken. The chicken had been artificially inoculated with five strains of *C. jejuni*. Recovery was poor with all the enrichment/plating combinations, but the enrichment media of Christopher et al. (1981) and Park et al. (1983) combined with Butzler's or CCDA media gave best results. The medium of Martin et al. (1983) performed particularly poorly. Preston broth and the medium of Rosef and Kapperud (1983) gave intermediate results. Direct plating of chicken samples diluted in 0.1% peptone water gave better recovery than enrichment, with CCDA and Campy-BAP yielding higher counts than Butzler agar. Preliminary study of Doyle and Roman's enrichment broth indicated that it was superior to other media.

Fricker (1984) undertook a comparison of three enrichment media: Preston, Lander and Doyle and Roman all incubated at 42°C, one transport/storage medium stored at 4°C (Campy-thio: Blaser et al. 1979) and two plating media – Preston and Campy-BAP. All media had 0.05% FBP added and were incubated aerobically in 6 ml quantities in quarter oz (*ca.* 7.5 ml) bottles. Naturally contaminated giblets from frozen chickens were examined. 177/198 samples were positive for campylobacters. Doyle and Roman and Preston media both detected 176 positive samples while Lander medium detected 159 and the Campy-thio medium 69. Samples were not plated directly, but the result using Campy-thio, in which multiplication of campylobacters would not have occurred, was probably similar to the result that would have been found by direct plating. Preston agar isolated campylobacters more frequently than Campy-BAP (577 v. 562 times). 48 h was the optimal time for incubation at 42°C. Enrichment in either Doyle and Roman or Preston broth followed by plating onto Preston agar was recommended.

A comparison of the Doyle and Roman enrichment method with that of Lovett et al. (1983) for isolation from milk was carried out by Hunt et al. (1985). The Lovett method was significantly better. Heisick et al. (1984) and Heisick (1985) also compared the Doyle and Roman broth with the Lovett et al. medium, using artificially inoculated beefburgers or milk-puddings and various strains of *C. jejuni*. The two methods gave similar results, although higher numbers of *C. jejuni* were sometimes achieved in the Lovett medium, especially when added in the absence of food.

Peterz (1991) conducted a collaborative trial amongst 6 laboratories in Scandinavia, testing chicken liver artificially inoculated with two strains of *C. jejuni* at levels between 0.3 and 59 per g. 2.5 g were enriched in 50 ml of Preston broth, without FBP but incubated microaerobically, at 42°C for 24 h, followed by plating onto mCCDA and Preston agar. A detection limit of about one campylobacter per g of liver was determined. The two plating media gave similar isolation rates, although mCCDA contained fewer contaminants.

De Boer and Humphrey (1991) compared Park and Sanders method to Preston

broth and mCCD broth for chilled or frozen chicken and found Park and Sanders superior, but gave no details. Exeter medium (modified from that described by Humphrey (1986b) see Table 3) was reported to give good results when used with a 37°C 18 h pre-enrichment step.

De Boer and Reitsma (1991) compared the effectiveness of Preston broth (without FBP) and mCCD broth in combination with plating onto Skirrow, Preston, mCCDA and filtration through an 0.6 μm pore membrane used with blood agar (modified Steele and McDermott, 1984). All incubation was microaerobic and naturally contaminated chilled and frozen chickens were examined. The two enrichment broths gave similar results, most positives occurring with mCCD broth plated via the membrane filter. Direct plating of frozen chicken gave much lower numbers of positive results than when samples were enriched (e.g. 8% positive versus 27% on mCCDA with or without enrichment in Preston broth respectively). With chilled chicken, direct plating on mCCDA gave 32% positive, compared with 47% positive after enrichment. Direct plating using the membrane filter method was completely ineffective.

The study of Furanetto et al. (1991), also examining naturally contaminated chilled chicken carcasses, compared direct plating on Campy-BAP or VTP-FBP agar with plating on the same media after enrichment in Doyle and Roman or Preston broth. All incubation was static in a micro-aerobic atmosphere. The combination of Doyle and Roman enrichment with Campy-BAP gave the highest number (16/42) of positive samples, although the results were not statistically significantly different from direct plating on either Campy-BAP (13/42) or VTP-FBP agar (11/42). The combination of Doyle and Roman broth with VTP-FBP agar gave particularly poor results (1/42).

Stern and Line (1992) compared the enrichment broths of Doyle and Roman, Park and Sanders and Hunt and Radle (plating onto Campy-BAP, mCCDA and Campy-Cefex) for examining whole chilled, naturally contaminated chickens. Inocula were prepared by rinsing the carcasses with 200 ml buffered peptone, filtering the rinse through cheesecloth, centrifuging, discarding the supernatant, streaking some of the pellet onto the selective agars, resuspending the rest of the pellet in 5 ml buffered peptone water and inoculating 1 ml of the resuspension to 100 ml of enrichment broth. Microaerobic atmosphere was obtained by replacing the air in polythene bags with the appropriate gas mixture (flushing 3 times). All bags were incubated in water baths with shaking. The Doyle and Roman broth was incubated at 42°C. The Park and Sanders and Hunt and Radle broths were incubated at 32°C for 3.5 h, 37°C for 2.5 h and then at 42°C. Cefoperazone supplement was added to the Hunt and Radle broth and cefoperazone and cycloheximide to the Park and Sanders broth before raising the temperature from 32 to 37°C. Re-gassing of the enrichment broths was carried out after adding the supplements. Ten out of 50 chicken carcasses were positive for campylobacter by direct plating. Forty-nine out of 50 were detected by all the enrichment/plating methods combined. 23, 40 and 43 positive chickens were detected using Doyle and Roman, Park and Sanders and Hunt and Radle broths respectively. mCCD agar gave most positive samples

overall, and with the Park and Sanders method, although all three selective media detected similar numbers of positive samples from Hunt and Radle broth.

The study of Scotter et al. (1993) used frozen samples of naturally contaminated chicken skins, in which the campylobacters were more likely to be damaged, and therefore require pre-enrichment. Twelve laboratories in the UK participated in the trial. Tests were carried out using two methods defined in a draft International Standards Organisation Method. The three enrichment methods used 10 g quantities of food in 90 ml of enrichment medium, under a small headspace. They were as follows: (1) Preston broth without FBP supplement, incubated in micro-aerobic atmosphere at 42°C for 48 h; (2) Park and Sanders broth incubated aerobically, with antibiotic supplement and at times and temperatures prescribed by Park and Sanders (1989); (3) in Preston broth containing FBP supplement, aerobically, but according to the temperatures and times prescribed by Humphrey (1989) – 37°C for 4 h and then 42°C for 44 h. All broths were plated directly onto Skirrow agar. Method 1 used any selective medium chosen by the participant, method 2 used a 0.65 μm membrane on non-selective blood agar and method 3 used Preston agar in addition. All plates were incubated at 42°C. At 10 cells per 10 g chicken skin all three methods were equally effective. At about 2 cells per 10 g, method 2 gave significantly higher isolation rates. Skirrow agar was frequently overgrown with pseudomonads and proteus. The membrane plus non-selective agar was as effective as selective agars such as mCCDA. No advantage was gained by pre-enriching at 37°C for 4 h (method 3). However, pre-enrichment at 37°C for 4 h was intended by Humphrey (1989) for use with his (Exeter) medium (Humphrey, 1986b), not Preston broth.

15. Conclusions

Media for isolating campylobacters and related bacteria from faeces and other environments such as food and water are not yet optimal, and there is so far no consensus concerning the best media and methods. The key to elucidating efficient and economic media and methods might lie in more investigation into the phenomenon of 'viable but not culturable' campylobacters and the ability of at least some strains of *C. jejuni* to adapt to grow in air, losing the ability to grow in a microaerobic atmosphere (Jones et al, 1993). Future developments could include the use of aerobic incubation and/or different media for some groups, as well as 37°C instead of 42° or 43°C. Some plating media are already incubated at 37°C.

Of the plating media listed in Table 2, probably the most widely used today are Skirrow, Campy-BAP, Preston, mCCD, Butzler (Virion) and Karmali. mCCD and Karmali agars have performed best in comparative studies using faecal samples. Other media – e.g. the semi-solid medium of Goossens et al. (1989) and the medium of Aspinall et al. (1993), have yet to be tested in comparative studies. If cefoperazone- and polymyxin- or colistin-sensitive campylobacters are sought and/or arcobacters and *C. jejuni* subsp. *doylei*, which do not grow at 42°C or 43°C, media such as mCCD, those of Goossens et al. (1986) and Aspinall et al. (1993)

and the semi-solid medium of Goossens et al. (1989) incubated at 37°C, might be useful. Use of modifications of the membrane filter method of Steele and McDermott (1984) in combination with non-selective blood agar are useful for this group, but only if relatively high numbers of campylobacters are present in the faecal suspension or enrichment culture.

Development of liquid enrichment media has so far been confined to the isolation of thermophilic campylobacters, predominantly *C. jejuni* subsp. *jejuni*. Future work may be directed towards modifying these media and current methods in order to detect the mesophilic and antibiotic-sensitive types mentioned above. This may, in turn, depend on whether those types are perceived to be an important cause of diarrhoea, and whether contaminated food is shown to be a vector.

More investigation is needed concerning the optimal atmospheric conditions necessary for thermophilic campylobacters in liquid medium, including whether an aerobic atmosphere might be advantageous for some applications, possibly in parallel with micro-aerobic conditions.

Acknowledgements

We would like to thank Margaret Smart for typing the manuscript and David Corry for translating the paper by Brennhovd and Kapperud (1991) from Norwegian.

References

Acuff, G.R., Vanderzant, C., Gardner, F.A. and Golan, F.A. (1982) Evaluation of an enrichment plating procedure for the recovery of *Campylobacter jejuni* from turkey eggs and meat. J. Food Prot. 45, 1276–1278.

Ahonkhai, V.I., Cherubin, C.E., Sierra, M.F., Bokkenheuser, V.D., Shulman, M.A. and Mosenthal, A.C. (1981) In vitro susceptibility of *Campylobacter fetus* subsp. *jejuni* to N-formimidoyl thienamycin, rosaramicin, cefoperazone and other antimicrobial agents. Antimicrob. Agents Chemother. 20, 850–851.

Albert, M.J., Tee, W., Leach, A.S., Asche, V. and Penner, J.L. (1992) Comparison of a blood-free medium and a filtration technique for the isolation of *Campylobacter* spp. from diarrhoeal stools of hospitalised patients in central Australia. J. Med. Microbiol. 37, 176–179.

Anon. (undated) Lab-M Culture Media. Lab-M, Bury BL9 6AU, UK, p. 36.

Aspinall, S.T., Wareing, D.R.A., Hayward, P.G. and Hutchinson, D.N. (1993) Selective medium for thermophilic campylobacters including *Campylobacter upsaliensis*. J. Clin. Pathol. 46, 829–831.

Barot, M.S. and Bokkenheuser, V.D. (1984) Systematic investigation of enrichment media for wild-type *Campylobacter jejuni* strains. J. Clin. Microbiol. 20, 77–80.

Baird, R.M., Corry, J.E.L. and Curtis, G.D., editors (1987) Pharmacopoeia of culture media for food microbiology. Int. J. Food Microbiol. 5, 212–213 (mCCD agar), 250–253 (Preston media), 268–269 (Skirrow agar).

Beuchat, L.R. (1985) Efficacy of media and methods for detecting and enumerating *Campylobacter jejuni* in refrigerated chicken meat. Appl. Environ. Microbiol. 50, 934–939.

Blaser, M.J., Cravens, J., Powers, R. and Wang, W.L. (1978) Campylobacter enteritis associated with canine infection. Lancet 2, 979–981.

Blaser, M.J., Berkowicz, I.D., Laforce, F.M., Cravens, J., Reller, L.B. and Wang, W.L. (1979) Campylobacter enteritis: clinical and epidemiological features. Ann. Intern. Med. 91, 179–185.

Blaser, M.J., Hardesty, H.L., Powers, B. and Wang, W.L.L. (1980) Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. J. Clin. Microbiol. 11, 309–313.

Bolton, F.J., Hinchcliffe, P.M., Coates, D. and Robertson, L. (1982) A most probable number method for estimating small numbers of campylobacters in water. J. Hyg. Camb. 89, 185–190.

Bolton, F.J. and Robertson, L. (1982) A selective medium for isolating *Campylobacter jejuni / coli*. J. Clin. Pathol. 35, 462–467.

Bolton, F.J. and Coates, D. (1983) Development of a blood-free campylobacter medium: screening tests on basal media and supplements, and the ability of selected supplements to facilitate aerotolerance. J. Appl. Bacteriol. 54, 115–125.

Bolton, F.J., Coates, D., Hinchcliffe, P.M. and Robertson, L. (1983) Comparison of selective media for isolation of *Campylobacter jejuni / coli*. J. Clin. Pathol. 36, 78–83.

Bolton, F.J., Coates, D. and Hutchinson, D.N. (1984a) The ability of *Campylobacter* media supplements to neutralise photochemically induced toxicity and hydrogen peroxide. J. Appl. Bacteriol. 56, 151–157.

Bolton, F.J., Hutchinson, D.N. and Coates, D. (1984b) A blood-free selective medium for the isolation of *Campylobacter jejuni* from faeces. J. Clin. Microbiol. 19, 169–171.

Bolton, F.J., Coates, D. Hutchinson, D.N. and Godfree, A.F. (1987) A study of thermophilic campylobacters in a river system. J. Appl. Bacteriol. 62, 167–176.

Bolton, F.J., Wareing, D.R.A., Skirrow, M.B. and Hutchinson, D.N. (1992) Identification and biotyping of campylobacters. In: Board, R.D., Jones, D. and Skinner, F.A. (Editors) Identification Methods in Applied and Environmental Microbiology. Society for Applied Bacteriology Technical Series, 29. Blackwell, Oxford, pp. 151–161.

Boudreau, M., Higgins, R. and Mittal, K.R. (1991) Biochemical and serological characterisation of *Campylobacter cryoaerophila*. J. Clin. Microbiol. 29, 54–58.

Brennhovd, O. and Kapperud, G. (1991) Comparison of three methods of isolation of thermotolerant *Campylobacter* spp. from water In: Brennhovd, O. (Editor) Termotolerante *Campylobacter* spp. og *Yersinia* spp. i noem norske vannforekomster. Ph.D. thesis. Norwegian College of Veterinary Medicine, Oslo (in Norwegian).

Brennhovd, O., Kapperud, G. and Langeland, G. (1992) Survey of thermotolerant *Campylobacter* spp. and *Yersinia* spp. in three surface water sources in Norway. Int. J. Food Microbiol. 15, 327–338.

Brooks, B.W., Garcia, M.M., Frazer, A.D.E., Lior, H., Stewart, R.B. and Lammerding, A.M. (1986) Isolation and characterisation of cephalothin-susceptible *Campylobacter coli* from slaughtered cattle. J. Clin. Microbiol. 24, 591–595.

Buck, G.E. and Kelly, M.T. (1981) Effect of moisture content of the medium on colony morphology of *Campylobacter fetus* subsp. *jejuni*. J. Clin. Microbiol. 14, 585–586.

Burnens, A.P. and Nicolet, J. (1992) Detection of *Campylobacter upsaliensis* in diarrheic dogs and cats, using a selective medium with cephoperazone. Am. J. Vet. Res. 53, 48–51.

Busse, M. (1994) Media for salmonellae. Int. J. Food Microbiol. in press.

Butzler, J.P., Dekeyser, P. and Detrain, M. (1973) Related *Vibrio* in stools. J. Pediatr. 82, 493–495.

Butzler, J.P., Dekeyser, P. and Lafontaine, T. (1974) Susceptibility of related vibrios and *Vibrio fetus* to twelve antibiotics. Antimicrob. Agents Chemother. 5, 86–89.

Butzler, J.P. and Skirrow, M.B. (1979) Campylobacter enteritis. Clin. Gastroenterol. 8, 737–765.

Butzler, J.P., De Boeck, M. and Goossens, H. (1983) New selective medium for isolation of *Campylobacter jejuni* from faecal specimens. Lancet i, 818.

Christopher, F.M., Smith, G.C. and Vanderzant, C. (1982) Examination of poultry giblets, raw milk and meat for *Campylobacter fetus* subsp. *jejuni*. J. Food Prot. 45, 260–262.

De Boer, E. and Humphrey, T.J. (1991) Comparison of methods for the isolation of thermophilic campylobacters from chicken products. Microb. Ecol. Health Dis. 4 (Special Issue) S43.

De Boer, E. and Reitsma, W. (1991) Isolation of *Campylobacter* from poultry products using membrane filtration. In: Ruiz-Palacios G.M., Calva, F. and Ruiz-Palacios, B.R. (Editors) Campylobacter V. Proc. 5th International Workshop on Campylobacter Infection, Instituto Nacional de la Nutricion, Vasco de Quiroga 15, Mexico 1400, D.F., pp. 113–115.

Dekeyser, P., Gossuin-Detrain, M., Butzler, J.P. and Sternon, J. (1972) Acute enteritis due to related vibrio: first positive stool cultures. J. Infect. Dis. 125, 390–392.

Doyle, M.P. and Roman, D.J. (1982) Recovery of *Campylobacter jejuni* and *Campylobacter coli* from inoculated foods by selective enrichment. Appl. Environ. Microbiol. 43, 1343–1353.

Ehlers, J.G., Chapparo-Serrano, M., Richter, R.L. and Vanderzant, C. (1982) Survival of *Campylobacter fetus* subsp. *jejuni* in cheddar and cottage cheese. J. Food Prot. 45, 1018–1021.

FDA (Food and Drug Administration) Bacteriological Analytical Manual (1992) 7th edition. AOAC, Arlington, VA.

Fraser, A.D.E., Chandan, V., Yamazaki, H., Brooks, B.W. and Garcia, M.M. (1992) Simple and economical culture of *Campylobacter jejuni* and *Campylobacter coli* in CO_2 in moist air. Int. J. Food Microbiol. 15, 377–382.

Fricker, C.R. (1984) Procedures for the isolation of *Campylobacter jejuni* and *Campylobacter coli* from poultry. Int. J. Food Microbiol. 1, 149–154.

Fricker, C.R. (1985) A note on the effect of different storage procedures on the ability of Preston medium to recover campylobacters. J. Appl. Bacteriol. 58, 57–62.

Fricker, C.R., Girdwood, R.W.A. and Munro, D. (1983) A comparison of procedures for the isolation of campylobacters from seagull faeces. J. Hyg. Camb. 91, 445–450.

Furanetto, S.M.P., Nascimento, D.D., Cerqueira-Campos, M.L. and Iaria, S.T. (1991) Efficacy of direct plating and selective enrichment media for detecting *Campylobacter jejuni* in fresh eviscerated whole market chickens – São Paulo, Brazil. Rev. Microbiol. São Paulo 22, 303–307.

Gebhart, C.J., Ward, G.E. and Kurtz, H.J. (1985) In vitro activities of 47 antimicrobial agents against three *Campylobacter* spp. from pigs. Antimicrob. Agents Chemother. 27, 55–59.

George, H.A., Hoffman, P.S., Smibert, R.M. and Krieg, N.R. (1978) Improved media for growth and aerotolerance of *Campylobacter fetus*. J. Clin. Microbiol. 8, 36–41.

Gilchrist, M.J.R., Grewell, C.M. and Washington, J.A. (1981) Evaluation of media for isolation of *Campylobacter fetus* subsp. *jejuni* from faecal specimens. J. Clin. Microbiol. 14, 393–395.

Goossens, H., De Boeck, M. and Butzler, J.P. (1983) A new selective medium for the isolation of *Campylobacter jejuni* from human faeces. Eur. J. Clin. Microbiol. 2, 389–394.

Goossens, H. De Boeck, M., Coignau, H., Vlaes, L., Van Den Borre, C. and Butzler, J.P. (1986) Modified selective medium for isolation of *Campylobacter* spp. from faeces: comparison with Preston medium and blood-free medium and a filtration system. J. Clin. Microbiol. 24, 840–845.

Goossens, H., Vlaes, L., Galand, I., Van Den Borre, C. and Butzler, J.P. (1989) Semisolid blood-free selective motility medium for the isolation of campylobacters from stool specimens. J. Clin. Microbiol. 27, 1077–1080.

Goossens, H., Pot, B., Vlaes, L. et al. (1990a) Characterisation and description of '*Campylobacter upsaliensis*' isolated from human faeces. J. Clin. Microbiol. 28, 1039–1046.

Goossens, H., Vlaes, L., DeBoeck, M. et al. (1990b) Is '*Campylobacter upsaliensis*' an unrecognised cause of human diarrhoea? Lancet 335, 584–586.

Gun-Munro, J., Rennie, R.P., Thornley, J.H., Richardson, H.L., Hodge, D. and Lynch, J. (1987) Laboratory and clinical evaluation of isolation media for *Campylobacter jejuni*. J. Clin. Microbiol. 25, 2274–2277.

Heisick, J. (1985) Comparison of enrichment broths for isolation of *Campylobacter jejuni*. Appl. Environ. Microbiol. 50, 1313–1314.

Heisick, J., Lanier, J. and Peeler, J. (1984) Comparison of enrichment methods and atmosphere modification procedures for isolating *Campylobacter jejuni* from foods. Appl. Environ. Microbiol. 48, 1254–1255.

Hoffman, P., Krieg, N.R. and Smibert, R.M. (1979a) Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. I. Physiological aspects of enhanced aerotolerance. Can. J. Microbiol. 25, 1–7.

Hoffman, P.S., George, H.A., Krieg, N.R. and Smibert, R.M. (1979b) Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. II. Role of exogenous superoxide anions and hydrogen peroxide. Can. J. Microbiol. 25, 8–16.

Höller, C. (1991) A note on the comparative efficacy of three selective media for isolation of *Campylobacter* species from environmental samples. Zbl. Hyg. Unweltmed. 192, 116–123.

Humphrey, T.J. (1986a) Injury and recovery in freeze-or-heat damaged *Campylobacter jejuni*. Lett. Appl. Microbiol. 3, 81–84.

Humphrey, T.J. (1986b) Techniques for optimum recovery of cold-injured *Campylobacter jejuni* from milk and water. J. Appl. Bacteriol. 61, 125–132.

Humphrey, T.J. (1989) An appraisal of the efficacy of pre-enrichment for the isolation of *Campylobacter jejuni* from water and food. J. Appl. Bacteriol. 66, 119–126.

Humphrey, T.J. and Cruikshank, J.G. (1985) Antibiotic and deoxycholate resistance in *Campylobacter jejuni* following freezing or heating. J. Appl. Bacteriol. 59, 65–71.

Hunt, J.M. (1992) Campylobacter. In: Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC, Arlington, VA, pp. 77–94

Hunt, J.M., Francis, D.W., Peeler, J.T. and Lovett, J. (1985) Comparison of methods for isolating *Campylobacter jejuni* from raw milk. Appl. Environ. Microbiol. 50, 535–536.

Hutchinson, D.N. and Bolton, F.J. (1984) Improved blood-free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J. Clin. Pathol. 37, 956–957.

Jacob, J. and Stelzer, W. (1992) Comparison of two media for the isolation of thermophilic campylobacters from waste waters of different quality. Zbl. Mikrobiol. 147, 42–44.

Jones, D.M., Sutcliffe, E.M. and Curry, A. (1991) Recovery of viable but non-culturable *Campylobacter jejuni*. J. Gen. Microbiol. 137, 2477–2482.

Jones, D.M., Sutcliffe, E.M., Ribs, R., Fox, A.J. and Curry, A. (1993) *Campylobacter jejuni* adapts to aerobic metabolism in the environment. J. Med. Microbiol. 38, 145–150.

Juven, B.J. and Rosenthal, I. (1985) Effect of free-radical and oxygen scavengers on photochemically generated oxygen toxicity and on the aerotolerance of *Campylobacter jejuni*. J. Appl. Bacteriol. 59, 413–419.

Karmali, M.A., De Grandis, S. and Fleming, P.C. (1980) Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus* to eight cephalosporins with special reference to species differentiation. Antimicrob. Agents Chemother. 18, 948–951.

Karmali, M.A., De Grandis, S. and Fleming, P.C. (1981) Antimicrobial susceptibility of *Campylobacter jejuni* with special reference to resistance patterns of Canadian isolates. Antimicrob. Agents Chemother. 19, 593–597.

Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S. and Lane, J. (1986) Evaluation of a blood-free, charcoal-based, selective medium for the isolation of *Campylobacter* organisms from faeces. J. Clin. Microbiol. 23, 456–459.

Kiehlbauch, J.A. Brenner, D.J., Nicholson, M.A., Baker, C.N., Patton, C.M., Steigerwalt, A.B. and Wachsmuth, I.K. (1991) *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrhoeal illness. J. Clin. Microbiol. 29, 376–385.

Kiehlbauch, J.A., Baker, C.N. and Wachsmuth, I.K. (1992) In vitro susceptibilities of aerotolerant *Campylobacter* isolates to 22 antimicrobial agents. Antimicrob. Agents Chemother. 36, 717–722.

Lander, K.P. (1982) A selective, enrichment and transport medium for campylobacters. In: D.G. Newell (Editor) Campylobacter: Epidemiology, Pathogenesis and Biochemistry. MTP Press, Lancaster, pp. 77–78.

Lastovica, A.J., Le Roux, E. and Penner, J.L. (1989) '*Campylobacter upsaliensis*' isolated from blood cultures of paediatric patients. J. Clin. Microbiol. 276, 657–659.

Lauwers, S. De Boeck, M. and Butzler, J.P. (1978) Campylobacter enteritis in Brussels. Lancet i, 604–605.

Lovett, J., Francis, D.W. and Hunt, J.M. (1983) Isolation of *Campylobacter jejuni* from raw milk. Appl. Environ. Microbiol. 46, 459–462.

Martin, W.H., Patton, C.M., Morris, G.K., Potter, M.E. and Puhr, N.D. (1983) Selective enrichment broth medium for isolation of *Campylobacter jejuni*. J. Clin. Microbiol. 17, 853–855.

Megraud, F. (1987) Isolation of *Campylobacter* spp. from pigeon faeces by a combined enrichment-filtration technique. Appl. Environ. Microbiol. 53, 1394–95.

Megraud, F. and Gavinet, A.M. (1987) Comparison of three protocols to isolate *Campylobacter* species from human faeces. In: Kaijser, B. and Falsen, E. (Editors) Campylobacter IV. Proc. 4th International Workshop on Campylobacter Infections, Univ. Göteborg, Sweden, pp. 90–91.

Merino, F.J., Agulla, A., Villasante, P.A., Díaz, A., Saz, J.V. and Velasco, A.C. (1986) Comparative efficacy of seven selective media for isolating *Campylobacter jejuni*. J. Clin. Microbiol. 24, 451–452.

Michel, J., Rogol, M. and Dickman, D. (1983) Susceptibility of clinical isolates of *Campylobacter jejuni* to sixteen antimicrobial agents. Antimicrob. Agents Chemother. 23, 796–797.

Moreno, G.S., Griffiths, P.L., Connerton, I.F. and Park, R.W.A. (1993) Occurrence of campylobacters in small domestic and laboratory animals. J. Appl. Bacteriol. 75, 49–54.

Morris, G.K., Bopp, C.A., Patton, C.M. and Wells, J.G. (1982) Media for isolating campylobacter. Arch. Lebensmittelhyg. 33, 151–153.

Ng, L.K., Stiles, M.E. and Taylor, D.E. (1985) Inhibition of *Campylobacter coli* and *Campylobacter jejuni* by antibiotics used in selective growth media. J. Clin. Microbiol. 22, 510–514.

Ng, L.K., Taylor, D.E. and Stiles, M.E. (1988) Characterisation of freshly isolated *Campylobacter coli* strains and suitability of selective media for their growth. J. Clin Microbiol. 26, 518–523.

Park, C.E., Stankiewicz, Z.K., Lovett, J. and Hunt, J. (1981) Incidence of *Campylobacter jejuni* in fresh eviscerated whole market chickens. Can. J. Microbiol. 27, 841–842.

Park, C.E. and Stankiewicz, Z.K. (1982) A selective enrichment procedure for the isolation of *Campylobacter jejuni* from foods. In: Newell, D.G. (Editor) Campylobacter: Epidemiology, Pathogenesis and Biochemistry. MTP, Lancaster, UK, p. 79.

Park, C.E., Stankiewicz, Z.K., Lovett, J., Hunt, J. and Francis, D.W. (1983) Effect of temperature, duration of incubation and pH of enrichment culture on the recovery of *Campylobacter jejuni* from eviscerated market chickens. Can. J. Microbiol. 29, 803–806.

Park, C.E. and Sanders, G.W. (1991) A sensitive enrichment procedure for the isolation of *Campylobacter jejuni* from frozen foods. In: Riuz-Palacios, G.M., Calva, F. and Ruiz-Palacios, B.R. (Editors) Campylobacter V, Proc. 5th International Workshop on Campylobacter Infections, National Institute of Nutrition, Puerto Vallarta, Mexico, p. 102.

Patton, C.M., Mitchell, S.W., Potter, M.E. and Kaufman, A.F. (1981) Comparison of selective media for primary isolation of *Campylobacter fetus* subsp. *jejuni*. J. Clin. Microbiol. 13, 326–330.

Patton, C.M., Shaffer, N., Edmonds, P. et al. (1989) Human disease associated with *Campylobacter upsaliensis* (catalase-negative or weakly positive *Campylobacter* species) in the United States. J. Clin. Microbiol. 27, 66–73.

Pearson, A.D. and Healing, T.D. (1992) The surveillance and control of campylobacter infection. Communicable Disease Report Review, 2. Public Health Laboratory Service, London, R133–140.

Pennie, R.A., Zurino, J.N., Rose, C.E. and Guerrant, R.L. (1984) Economical simple method for production of the gaseous environment required for cultivation of *Campylobacter jejuni*. J. Clin. Microbiol. 20, 320–322.

Peterz, M. (1991) Comparison of Preston agar and a blood-free selective medium for detection of *Campylobacter jejuni* in food. J. Assoc. Off. Anal. Chem. 74, 651–654.

Plastridge, W.N., Williams, L.F. and Trowbridge, D.G. (1964) Antibiotic sensitivity of physiologic groups of microaerophilic vibrios. Am. J. Vet. Res. 25, 1295–1299.

Ray, B. and Johnson, C. (1984a) Sensitivity of cold-stressed *Campylobacter jejuni* to solid and liquid enrichments. Food Microbiol. 1, 173–176.

Ray, B. and Johnson, C. (1984b) Survival and growth of freeze-stressed *Campylobacter jejuni* cells in selective media. J. Food Safety 6, 183–195.

Razi, M.H.H. and Park, R.W.A. (1979) Studies of media for the isolation and storage of *Campylobacter* spp. J. Appl. Bacteriol. 47, x.

Ribeiro, C.D. and Price, T.H. (1984) The use of Preston enrichment broth for the isolation of 'thermophilic' campylobacters from water. J. Hyg., Camb. 92, 45–51.

Ribeiro, C.D., Marks, J. and Grimshaw, A.D. (1985) Economic cultivation of 'thermophilic' *Campylobacter* spp. J. Clin. Pathol. 38, 1311–1313.

Rosef, O. (1981) Isolation of *Campylobacter fetus* subsp. *jejuni* from the gall bladder of normal slaughter pigs, using an enrichment procedure. Acta Vet. Scand. 22, 149–151.

Rosef, O. and Kapperud, G. (1983) House flies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. Appl. Environ. Microbiol. 45, 381–383.

Rosef, O., Gondrosen, B., Kapperud, G. and Underdal, B. (1983) Isolation and characterisation of *Campylobacter jejuni* and *Campylobacter coli* from domestic animals and wild animals in Norway. Appl. Environ. Microbiol. 46, 855–859.

Rosef, O., Kapperud, G. and Skjerve, E. (1987) Comparison of media and filtration procedures for qualitative recovery of thermotolerant *Campylobacter* spp. from naturally contaminated surface water. Int. J. Food Microbiol. 5, 29–39.

Scotter, S.L., Humphrey, T.J. and Henley, A. (1993) Methods for the detection of thermotolerant campylobacters in foods: results of an inter-laboratory study. J. Appl. Bacteriol. 74, 155–163.

Shanker, S., Gordon, S.W., Fuller, H. and Gilbert, G.L. (1991) Sensitivity of a filtration method for the detection of *Campylobacter / Helicobacter* spp. in faeces. Microb. Ecol. Health Dis. 4, S47.

Skirrow, M.B. (1977) Campylobacter enteritis: a new disease. Brit. Med. J. 2, 9–11.

Skirrow, M.B., Benjamin, J., Razi, M.H.H. and Waterman, S. (1982) Isolation, cultivation and identification of *Campylobacter jejuni* and *Campylobacter coli*. In: Corry, J.E.L., Roberts, D. and Skinner, F.A. (Editors) Isolation and Identification Methods for Food Poisoning Organisms. Academic Press, London, pp. 313–328.

Skirrow, M.B., Vickery, C.R., Lovelock, M.R. and Evans, J.J. (1987) Cheap microaerobic atmospheres: modification of the methylated spirit burn method. In: Kaijser, B. and Falsen, E. (Editors) Campylobacter IV, Proc. 4th International Workshop on Campylobacter Infections, Univ. Göteborg, Sweden.

Skirrow, M.B., Merry, T.J. and Vickery, C.R. (1991) Hydrogen facilitates the isolation of *Campylobacter jejuni*. In: Ruiz-Palacios, G.M., Calva, E. and Ruiz-Palacios, B.R. (Editors) Campylobacter V, Proc. 5th International Workshop on Campylobacter Infections, Instituto Nacional de la Nutricion, Vasco de Quiroga 15, Mexico 1400, D.F., p. 109.

Steele, T.W. and McDermott, S.W. (1984) The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from faeces. Pathology 16, 263–265.

Stelzer, W. and Jacob, J. (1992) Das Vorkommen von *Campylobacter* in einem Mittelgebirgsbach. Zbl. Mikrobiol. 147, 45–50.

Stelzer, W., Mochmann, H.P., Richter, U. and Dobberkau, H.J. (1988) Characterisation of *Campylobacter jejuni* and *Campylobacter coli* isolated from waste water. Zbl. Bakteriol. Hyg. A 269, 188–196.

Stern, N.J., Green, S., Thaker, N., Krout, D. and Chju, H. (1984) Recovery of *Campylobacter jejuni* from fresh and frozen meat and poultry collected at slaughter. J. Food Prot. 47, 372–374.

Stern, N.J. and Line, J.E. (1992) Comparison of three methods for recovery of *Campylobacter* spp. from broiler carcasses. J. Food Prot. 55, 663–666.

Stern, N.J., Wojton, B. and Kwiatek, K. (1992) A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55, 514–517.

Taylor, D.N., Kiehlbauch, J.A., Tee, W., Pitarangsi, C. and Echeverria, P. (1991) Isolation of group 2 aerotolerant *Campylobacter* species from Thai children with diarrhoea. J. Infect. Dis. 163, 1062–1067.

Tenova, F.C. and Fennell, C.L. (1992) The genera *Campylobacter* and *Helicobacter*. In: Balows, A., Truper, H.G., Dworkin, M., Hurder, W. and Schleifer, K.H. (Editors) The Prokaryotes IV, 2nd edition. Springer, New York, pp. 3488–3511.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. et al. (1991) Revision of *Campylobacter*, *Helicobacter* and *Wolinella* taxonomy: amendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int. J. Syst. Bacteriol. 41, 88–103.

Vandamme, P. and Goossens, H. 1992 Taxonomy of campylobacter, arcobacter and helicobacter. Zbl. Bakteriol. 276, 447–472.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D. et al. (1992a) Polyphasic taxonomic study of the amended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowi* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. Int. J. Syst. Bacteriol. 42, 344–356.

Vandamme, P., Pugina, P., Benzi, G., Van Etterijck, R., Vlaes, L., Kersters, K. et al. (1992b) Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. J. Clin. Microbiol. 30, 2335–2337.

Vanhoof, R., Vanderlinden, M.P., Diereckx, R., Lauwers, S., Yourassowsky, F. and Butzler, J.P. (1978) Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty nine antimicrobial agents. Antimicrob. Agents Chemother. 14, 553–556.

Walder, M. (1979) Susceptibility of *Campylobacter fetus* subsp. *jejuni* to twenty antimicrobial agents. Antimicrob. Agents Chemother. 16, 37–39.

Warmsley, S.L. and Karmali, M.A. (1989) Direct isolation of atypical thermophilic *Campylobacter* species from human faeces on selective agar medium. J. Clin. Microbiol. 27, 668–670.

Waterman, S.C., Park, R.W.A. and Bramley, A.J. (1984) A search for the source of *Campylobacter jejuni* in milk. J. Hyg., Camb. 92, 333–337.

Weber, G., Manafi, M. and Reisinger, H. (1987) Die Beduntung von *Yersinia enterocolitica* und thermophilen Campylobactern für die Wasserhygiene. Zbl. Bakteriol. Hyg. B 184, 501–514.

Weinrich, K., Winkler, K. and Heberer, E. (1990) Untersuchungen zur Eignung ausgewählter Huminsaurenchargen als Nahbodenzusatz für Medien sur Isolierung thermophiler Campylobacterspecies. Deusche Tierarztliche Wochenschrift 97, 511–515.

Wesley, R.D., Swaminathan, B. and Stadelman, W.J. (1983) Isolation and enumeration of *Campylobacter jejuni* from poultry products by a selective enrichment method. Appl. Environ. Microbiol. 46, 1097–1102.

US005891709A

United States Patent [19]

Stern et al.

[11] **Patent Number: 5,891,709**

[45] **Date of Patent: Apr. 6, 1999**

[54] **CAMPY-CEFEX SELECTIVE AND DIFFERENTIAL MEDIUM FOR CAMPYLOBACTER**

[75] Inventors: **Norman J. Stern**, Athens, Ga.; **Boleslaw J. Wojton**; **Kris Kwiatek**, both of Pulawy, Poland

[73] Assignee: **The United States of America as represented by the Secretary of the Agriculture**, Washington, D.C.

[21] Appl. No.: **912,447**

[22] Filed: **Jul. 13, 1992**

[51] **Int. Cl.**[6] **C12N 1/04**; C12N 1/12; C12N 1/20

[52] **U.S. Cl.** **435/252.1**; 435/260; 435/287.1; 435/822

[58] **Field of Search** 435/252.1, 253, 435/287, 260, 287.1, 822

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,904,597 2/1990 Inoue et al. 435/252.1

OTHER PUBLICATIONS

Hutchinson, D N, et al., "Is Enrichment Culture Necessary for the Isolation of *Campylobacter jejuni* from Faeces?", *J. Clin Pathol*, 1983, 36, pp. 1350–1352.

Hutchinson, D N, et al., "Improved Blood Free Selective Medium for the Isolation of *Campylobacter jejuni* from Faecal Specimens," *J. Chem. Pathol.*, 36, 1984, pp. 956–957.

Bolton, F J., et al., "A Selective Medium for isolating *Campylobacter jejuni/coli* ", *J. Clin Pathol*, 1982, 35, pp. 462–467.

Blaser, Martin J., MD, et al., "*Campylobacter enteritis*: Clinical and Epidemiologic Features", *Annals of Internal Medicine*, Aug. 1979, vol. 91, No. 2, pp. 179–185.

Rothenberg, P J, et al., "Selected Enrichment Broths for Recovery of *Campylobacter jejuni* from Foods", *Applied and Environmental Microbiology*, Jul. 1984, vol. 48, No. 1, pp. 78–80.

Hunt, Jan M., "Chapter 7—*Campylobacter*", in:*Food and Drug Administration Bacteriological Analytical Manual*, 7th Edition, 1992, (AOAC International), pp. 77–94.

Difco Laboratories, "Dehydrated Culture Media", *Difco Product Catalog and Reference Guide*, Effective: Jan. 1992, pp. 7–8.

"Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood", *Manual of BBL Products and Laboratory Procedures*, Sixth Edition, Power, David A., and McCuen, Peggy J., Editors, pp. 126–127 1992.

Smibert, Robert M., "Campylobacter", *Bergey's Manual of Systematic Bacteriology*, vol. 1, (Williams and Wilkins), pp. 111–115 1992.

Abstracts of Papers Presented at the Seventy–Eighth Annual Meeting of the IAMFES, *Journal of Food Protection*, Jul. 21–24, 1991, Louisville, KY, Oct. 1991, pp. 818–819.

Primary Examiner—Michael G. Wityshyn
Assistant Examiner—Deborah K. Ware
Attorney, Agent, or Firm—M. Howard Silverstein; Randall E. Deck; John D. Fado

[57] **ABSTRACT**

A solid or semi-solid culture medium, designated Campy-Cefex, for the isolation of Campylobacter species. The culture medium includes:

(a) a nutrient medium with an energy source effective to support growth of Campylobacter;

(b) agar;

(c) blood;

(d) a first selective agent selected from cycloheximide, its salts, or mixtures thereof; and

(e) a second selective agent selected from cefoperazone, its salts, or mires thereof.

In use, the sample to be analyzed is inoculated onto the Campy-Cefex culture medium, and subsequently incubated for a sufficient time and under conditions effective to promote growth of Campylobacter. Following incubation, the culture medium may then be examined for the presence of any colonies of Campylobacter.

8 Claims, No Drawings

CAMPY-CEFEX SELECTIVE AND DIFFERENTIAL MEDIUM FOR CAMPYLOBACTER

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to an improved medium for the selective and differential culture of Campylobacter species, espccially *C. jejuni* and *C. coli.*

2. Description of the Prior Art

Campylobacter jejuni, C. coli and *C. laridis* are known to cause an estimated 2.2 million cases of foodborne gastroenteritis per year in the United States alone (Tauxe et al., 1987, Am. J. Public Health, 77:1219–1221). The vast majority of these cases are associated with the consumption of improperly prepared poultry or foods and hands cross-contaminated by raw poultry. Although the origin of this disease in humans is primarily linked to poultry, the food microbiology and poultry communities have been slow in directing substantive attention toward the organism. In part, this has been due to the unique physiological requirements of these organisms, impairing their culture and identification from foods and clinical specimens.

A variety of enrichment and culture media have been proposed for the isolation of Campylobacter species [Park et al, Campylobacter, In: *Compendium of Methods for the Microbiological Examination of Foods*, second edit., M. L. Speck (ed.), Am. Pub. Hlth. Assoc., Washington, D.C., 1984, p. 386–404, the contents of which are incorporated by reference herein]. Because Campylobacter can be overgrown by other organisms present in sect sources, the use of selective media, incorporating antibiotics and/or antimicrobial agents, is essential for their culture. Ideally, any culture medium selected should also be differential, allowing the characterization of the Campylobacter by distinctive colonial appearances in culture.

Butzler developed a selective medium for *C. jejuni* containing a nutrient agar base, blood, and five selective agents, cycloheximide, cefazolin, bacitracin, colistin sulfate and novobiocin, as described by Smihert [Canpylobacter, In: *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Krieg and Holt (ed.), Williams and Wilkins, Baltimore, Md., 1984, pages 111–115, the contents of which are incorporated by reference herein]. Other selective media that have been developed include: Preston medium (Hut hinson and Bolton, J. Clin. Pathol., 1983, 36:1350–1352) containing nutrient hroth, agar, lysed horse hlood, cyclohemimide, polymyxin sulphate, trimethoprim lactate and rifampicin; Skirrow's medium (Skirrow, Br. Med. J., 1977, ii:9–11) containing a nutrient agar base, lysed horse blood, vancomycin, polymyxin and trimethoprim; and Campy-BAP (Blaser et al., 1979, Ann. Intern. Med., 91:179–185) containing brucella agar, sheep erythrocytes, vanoocycin, trimethoprim, polymyxin B, cephalothin, and amphotericin B. While all of these media offer some selectivity, the degree of this selectivity has been limited, and the growth of some strains of Campylobacter may be inhibited as well.

One agar medium in particular, CCDA, has gained prominence for the isolation of Campylobacter. Early formulations of CCDA (Hutchinson and Bolton, 1983, J. Clin. Pathol., 36:1350–1352) contained nutrient broth, agar, charcoal, casein hydrolysates, ferrous sulfate, sodium pyruvate and, as selective agents, sodium deoxyoholatc and cephazolin. In later formulations the cephazolin was replaced with cefoperazone for improved selectivity (Hutchinson and Bolton, 1984, J. Clin. Pathol., 37:956–957). However, colonies of

Campylobacter grown on CCDA have been difficult to differentiate from colonies of other containing microorganisms

SUMMARY OF THE INVENTION

We have now discovered an improved solid or semi-solid culture medium, designated Campy-Cefex, for the isolation of Campylobacter species. The culture medium includes:

(a) a nutrient medium with an energy source effective to support growth of Campylohacter;

(h) agar;

(c) blood;

(d) a first selective agent selected from cycloheximide, its salts, or mixtures thcreof; and

(e) a second selective agent selected from cefoperazone, its salts, or mixtures thereof.

In use, the sample to be analyzed is inoculated onto the Campy-Cefex culture medium, and subsequently incubated for a sufficient time and under conditions effective to promotc growth of Campylobacter. Following incubation, the culture medium may then be examined for the presence of any colonies of Campylohacter.

In accordance with this discovery, it is an object of this invention to provide an improved culture medium and method for the isolation of Campylobacter species.

A further object of this invention to provide a culture medium which is both selective and differential for the growth of Campylobacter in samples containing a mixed flora of microorganisms.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

DETAILED DESCRIPTION OF THE INVENTION

The Campy-Cefex medium of this invention may he used for the isolation of Campylohacter, particularly *C. jejuni* and/or *C. coli*, from a variety of sources. Although the mcdium is particularly valuable for the growth and recovcry of this microorganism from samples taken from poultry carcasses, especially chickens, it is understood that the medium may also he used for the isolation of Campylobacter from any samples suspected of containing this pathogen. Without being limited thereto, other sources include animal carcasses such as cattle and sheep, food, milk, water, or clinical sources such as faces or hlood. The culture medium contemplated for use in this invention may he prepared using techniques conventional in the art. The basal medium components, including agar and nutrient medium with an energy source are mixed and sterilized by autoclaving. After cooling the sterilized basal medium to about 50°–55° C., hlood and filter-sterilized cycloheximide and cefoperazone are added with mixing, and the medium finally poured into a culture container such as a petri dish or flask and cooled to allow the agar to solidify.

Basal medium components selected for use are not critical and may he readily determined by the practitioner skilled in the art. Any nutrient medium and energy source effective to support growth of Campylohacter, particularly *C. jejuni* and/or *C. coli*, may be used. Suitable nutrient media include hut are not limited to Brucella agar (e.g. BBL, Cockeysville, Md.; Difco Laboratories, Detroit, Mich.; or CM 691, Oxoid, Columhia, Md.), Campylobacter agar base (Difco), Blood agar base no. 2 (Oxoid), Brain-heart infusion agar (BBL; Difco), or Columhia Blood Agar Base. A variety of energy sources may also be employed, and may be incorporated into

commercially available nutrient media or added separately. Suitable energy sources for use in the medium are described by Smibert (in Bergey's Manual, ibid, the contents of which are incorporated by reference herein) and include pyruvate, citrate, succinate, cis-aconitate, isocitrate, α-ketoglutarate, fumarate, malate, and oxaloacetate.

The source of the blood added to the medium also is not critical. While horse blood, and especially lysed horse blood, is preferred, it is understood that other blood sources may be used, such as sheep blood.

As noted hereinabove, Campy-Cefex includes two selective agents to prevent the growth of contaminating microorganisms present in the samples to be tested, but which do not inhibit growth of Campylobacter species. The first selective agent is selected from cycloheximide, its salts, and mixtures thereof. The second selective agent is selected from cefoperazone, its salts, and mixtures thereof. Particularly preferred as the selective agents are the sodium salts of cycloheximide and cefoperazone. Surprisingly, we have discovered that the combination of these two selective agents in Campy-Cefex provides excellent selectivity for Campylobacter without the need for additional antibiotics or antibacterial agents employed by other media conventional in the art as described hereinabove.

Other adjuvants may also be incorporated into Campy-Cefex medium for enhancing growth and/or aerotolerance of Campylobacter. Preferred adjuvants enhancing aerotolerance are described by Smibert (ibid) and include but are not limited to sodium pyruvate, ferrous sulfate, bovine superoxide dismutase, catalase, and reducing agents such as sodium bisulfite or sodium metabisulfite. Particularly preferred for addition to the medium are ferrous sulfate, sodium metabisulfite or sodium bisulfite, and sodium pyruvate (FBP). It is understood that the use of blood in the media also enhances aerotolerance because it contains catalase and superoxide dismutase.

The concentration and amount of each of the components of the Canny-Cefex medium are variable and may be readily determined by the practitioner skilled in the art. The amount of each component of the basal or nutrient media should be effective to promote growth of Campylobacter species, while the amount of the first and second selective agents should be effective to inhibit growth of contaminating (non-Campylobacter) micro-organisms without substantially inhibiting growth of Campylobacter species relative to culture medium lacking these selective agents. Without being limited thereto, preferred ranges of the selective agents include about 20–50 mg of cefoperazone, and about 100–400 mg of cycoheximide per liter of Campy-Cefex medium. In accordance with a particularly preferred formulation of Campy-Cefex, ranges of the amount of each component per liter include but are not limited to:

Brucella agar	about 40–50 g
lysed horse blood	about 20–100 ml (2–10%)
sodium cycloheximide	about 100–400 mg
sodium ceforerazone	about 20–50 mg
ferrous sulfate	about 0.1–1 g
sodium bisulfite	about 0.05–5 g
sodium pyruvate	about 0.1–1.0 g, and
dH_2O	about 950 mL

The final pH of the medium should generally be between about 6.5 to 7.5.

In use, the sample to be analyzed is inoculated onto the culture medium using techniques conventional in the art and is incubated for a sufficient time and under conditions effective to promote growth of Campylobacter, especially *C. jejuni* and/or *C. coli*. Suitable conditions may be readily determined by the practitioner skilled in the art and are described by Smibert (ibid). Without being limited thereto, preferred conditions include a temperature between about 35° to 44° C., especially 42° to 43° C., and a low oxygen tension (i.e. microaerobic), especially an oxygen concentration between 3–6%. Techniques for generating this reduced atmosphere are well known in the art and are described, for example, by Inouc (U.S. Pat. No. 4,904,597), or Hutchinson and Bolton (J. Clin. Pathol., 1983, 36:1350–1352), or Park et al (ibid), the contents of each of which are incorporated by reference herein. In the alternative, a suitable atmosphere may be generated using dry ice placed in a sealed container with the cultures.

Following incubation, generally after about 24 to 72 hours, the culture may be examined for the presence of colonies indicative of Campylobacter. Colonies of Campylobacter on the Campy-Cefex medium of this invention are translucent and grow to approximately 2 to 5 mm in diameter, but may also grow to confluence if the medium is moist. These colonies can be readily discriminated from non-Campylobacter breakthrough flora, which are opaque in appearance. Further confirmatory testing of the colonies and speciation can be conducted as described by Park et al (ibid) or Smibert (ibid).

EXAMPLE 1

The Campy-Cefex medium of this invention was compared with two other culture media, Campy-BAP and a modified CCDA supplemented with cycloheximide, for their ability to isolate Campylobacter species from chicken carcasses.

Media: The composition of Campy-Cefex, modified CCDA and Campy-BAP are provided in Table 1. All media were prepared in our laboratories and used immediately or stored in the dark at 4° C. no more than two weeks prior to use. The basal media components were autoclaved at 121° C. for 15 min. The sterilized agar was then tempered in a waterbath to 50°–55° C., and the lysed horse blood and filter-sterilized antibiotics were added and gently mixed before the plates were poured. Filter-sterilized sodium cycloheximide (200 mg/L) was added as a 10% solution in 50% methanol. One gram of sodium cefoperazone was dissolved in 10 ml of sterile, distilled water. Each ml of this solution provided 100 mg cefoperazone/3 L of Campy-Cefex agar. The unused portion of cefoperazone was held in storage at −20° C.

Sampling: During the first stage of the experiment, 21 poultry carcasses were sampled, employing a carcass swab technique to obtain the carcass-associated microflora. The swab samples were acquired by pre-moistening sterile cotton swabs in Cary-Blair transport medium (Park et al., ibid, the contents of which are incorporated by reference herein) and swabbing a 100 cm^2 area in the vicinity of the chicken vent and inner thighs, after evisceration but before chilling of the carcasses. Swabs were put back into the tubes of Cary-Blair and transported on ice to the laboratory within two hours. Each swab was then plated onto each of the three media immediately upon arrival.

The swab samples were used to inoculate the three test media in parallel. After the swab was used to inoculate a corner of one test medium, it was rotated one-third turn to provide uniformity of sample application. The swabbed corner of the plate was streaked for isolation of the representative flora. Plates were assessed for the presence of

Campylobacter spp., and non-Campylobacter breakthrough flora, after incubation in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) at 42° C. for 48 hours, as previously described (Park et al., ibid). This atmosphere was obtained by flushing the normal atmosphere out of the container with the microaerobic atmosphere. Criteria for presumptive identification were based upon a translucent colonial appearance (on Campy-EAP and Campy-Cefex), microscopic examination for curved to spiral-shaped bacterial rods, and positive catalase and oxidase testing as described by Part et al. (ibid).

In the second stage of the study, two lots of ten, whole raw broiler carcasses were procured from a retail grocer. Each lot was comprised of carcasses produced by three or four different poultry companies. These carcasses were washed by shaking for one minute in large plastic bags containing 200 ml of sterile buffered peptone (pH 7.2) to obtain a representative, carcass-associated microflora. The wash water suspension was centrifuged at 5,000×g for 10 min and the pellet was resuspended in one-ml of buffered peptone. The suspension was swabbed and streaked on the three agar media as described above.

Results: Table 2 contains information comparing the efficiency of Campy-BAP, Campy-fex, and modified CCDA media in isolating Campylobacter spp. from chicken carcasses. Among the total of 41 carcasses tested, the organism was isolated 33 times using Campy-BAP; 37 times using Campy-Cefex; and 35 timcs using modificd CCDA. However, the Campy-BAP medium was not as selective as the other media. Contaminating breakthrough flora occurred consistently on the Campy-BAP medium and less frequently or not at all on the other two media tested. Although the modified CCDA medium exhibited similar selectivity when cared with Campy-Cefex medium, differentiation between Campylobacter colonics and other breakthrough flora was significantly more difficult on modified CCDA medium. The Campy-Cefex medium described herein provides both excellent selectivity and a capacity for differentiating Campylobacter species from breakthrough flora.

It is understood that the foregoing detailed description is given merely by way of illustration and that modifications and variations may bc made therein without departing from the spirit and scope of the invention.

TABLE 1

Composition of Agar Media.

Campy-Cefex agar.		
	Basal medium	
Brucella agar		44 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)		0.5 g
Sodium bisulfite		0.2 g
Sodium pyruvate		0.5 g
dH_2O		950 ml
	Supplements	
Sodium cefoperazone		33 mg
Sodium cycloheximide		200 mg
Lysed horse blood		50 ml
Modified CCDA agar.		
	Basal medium	
Nutrient broth No. 2		25 g
Bacteriological charcoal		4 g
Casein hydrolysate		3 g
Sodium desoxycholate		1 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)		0.25 g
Sodium pyruvate		0.25 g
Agar		12 g

TABLE 1-continued

Composition of Agar Media.

Yeast extract		2 g
dH_2O		1000 ml
	Supplements	
Sodium cefoperazonc		30 mg
Sodium cycloheximide		100 mg
Campy-BAP		
	Basal medium	
Brucella agar		44 g
dH_2O		950 ml
	Supplements	
Lysed horse blood		50 ml
Vancomycin		10 mg
Polymyxin B		2500 IU
Trimethoprim lactate		25 mg
Amphotericin B		2 mg
Cephalothin		15 mg

TABLE 2

Recovery of Campylobacter spp. (*C. jejuni, C. laridis*) from chicken carcasses by direct plating onto Campy-BAP, Campy-Cefex, and modified CCDA media.

Medium	Number of Carcasses Examinetd	Incidence-number of Campylobacter spp. positive carcasses	Contamination by other species Heavy[1]	Slight[2]
TRAIL 1 - post evisceration, pre-chilled carcasses				
Campy-BAP	21	16	4	17
Campy-Cefex	21	21	0	4
Modified CCDA	21	21	0	3
TRAIL 2 - carcasses procured from retail markets				
Campy-BAP	10	7	10	0
Campy-Cefex	10	7	6	0
Modified CCDA	10	7	3	1
TRIAL 3 - carcasses procured from retail markets				
Campy-BAP	10	10	10	0
Campy-Cefex	10	9	0	2
Modified CCDA	10	7	0	0

[1]less than 10% of the bacterial population on the plate
[2]greater than 10% of the bacterial population on the plate

We claim:

1. A composition for the isolation of Campylobacter species comprising:

(a) a nutrient medium with an energy source effective to support growth of *Campylobacter jejuni* or *Campylobacter coli* or both;

(b) agar;

(c) blood;

(d) a first selective agent selected from the group consisting of cycloheximide, salts of cycloheximide and mixtures thereof; and

(e) a second selective agent selected from the group consisting of cefoperazone, salts of cefoperazone, and mixtures thereof;

and wherein said composition does not include bacitracin, colistin sulfate or novobiocin as selective agents.

2. A composition as described in claim 1 wherein the concentration of said blood is between about 2% to 10%, the concentration of said first selective agent is between about 100 to 400 mg/liter, and the concentration of said second selective agent about 20 to about 50 mg/liter.

3. A composition as described in claim 1 wherein said energy source is selected from the group consisting of

pyruvate, citrate, succinate, cis-aconitate, isocitrate, α-ketoglutarate, fumarate, malate, and oxaloacetate.

4. A composition as described in claim **1** which includes ferrous sulfate, a reducing agent, and sodium pyruvate.

5. A composition as described in claim **4** wherein said reducing agent is selected from the group consisting of sodium bisulfite and sodium metabisulfite.

6. A composition as described in claim **1** wherein said blood is selected from the group consisting of horse blood and sheep blood.

7. A composition as described in claim **6** wherein said blood is lysed.

8. A composition for the recovery of Campylobacter species comprising:

(a) a nutrient medium;

(b) agar;

(c) ferrous sulfate;

(d) a reducing agent selected from the group consisting of sodium metabisulfite and sodium bisulfite;

(e) pyruvate;

(f) blood;

(g) a first selective agent selected from the group consisting of cycloheximide, salts of cycloheximide and mixtures thereof; and

(h) a second selective agent selected from the group consisting of cefoperazone, salts of cefoperazone, and mixtures thereof;

and wherein said composition does not include bacitracin, colistin sulfate or novobiocin as selective agents.

* * * * *